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DIGITAL PATHOLOGY AND BIOINFORMATICS ANALYSIS OF PIT1 EXPRESSION IN PITUITARY MACROADENOMAS

ABSTRACT: Introduction: Pituitary macroadenomas pose a challenge in clinical endocrinology due to their impact on hormonal balance and subsequent clinical complications. Traditional diagnostic methods often suffer from subjectivity, highlighting the need for a more objective approach.

Materials and Methods: This study was conducted as a retrospective secondary analysis of publicly available, de-identified data. Digital histopathological images were obtained from a digital pathology repository, while RNA-seq data, including PIT1 gene expression, were retrieved from the NCBI GEO database. Convolutional neural networks (CNN) were applied for tumor tissue segmentation and classification, while differential expression analysis was performed using DESeq2.

Results: The model achieved an accuracy of 92.3% in identifying tumor regions, while bioinformatics analysis revealed a significant upregulation of PIT1 expression in adenomas with more pronounced clinical symptoms ($log2FC = 1.8$, $p < 0.01$). Integrated analysis confirmed a strong correlation between morphological patterns and PIT1 expression levels, while regression analysis indicated that this gene is an independent predictor of clinical outcomes.

Discussion and Conclusion: The integration of digital pathology and bioinformatics analysis has shown promise in improving the diagnosis and classification of pituitary macroadenomas, paving the way for personalized therapy. Further studies on more heterogeneous samples could further validate the utility of this multidisciplinary approach.

Keywords: digital pathology, bioinformatics, PIT1, macroadenomas, pituitary.

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INTRODUCTION

Pituitary macroadenomas represent a significant challenge in clinical endocrinology due to their ability to influence hormone production, which can lead to various metabolic and clinical complications [1]. Traditional diagnostic methods, based on classical histopathological analysis, are often limited by the subjectivity of interpretation, potentially resulting in inconsistencies in tumor characterization [2]. Over the past few decades, the development of digital pathology has opened new avenues for objective and faster histopathological slide analysis, enabling the quantitative assessment of tumor morphological and molecular characteristics [1,3].

Given the increasing importance of molecular markers in identifying adenoma subtypes, the expression of the transcription factor PIT1 has gained significance. PIT1 is crucial for pituitary hormone regulation, and its dysregulated expression has been associated with specific pathophysiological processes in macroadenomas [2,4]. The application of bioinformatics tools, particularly RNA-seq data analysis, allows for detailed mapping of PIT1 expression and the identification of patterns that may be useful in distinguishing benign from more aggressive adenoma subtypes.

The integration of digital pathology and bioinformatics analysis represents an innovative approach that promises to enhance the accuracy of pituitary macroadenoma diagnosis and classification. This multidisciplinary strategy not only contributes to a better understanding of tumor biology but also paves the way for personalized therapy tailored to the specific molecular profile of each patient [1,3,4].

Research Questions:

1. Does the integrated application of digital pathology and bioinformatics analysis of PIT1 expression enable more precise diagnosis and classification of pituitary macroadenomas compared to traditional methods?

2. What are the correlation patterns between PIT1 expression levels and clinical outcomes (such as prognosis and treatment response) in patients with pituitary macroadenomas?

3. How can a digitized approach to histopathological image analysis contribute to the standardization of diagnostic criteria in neuroendocrine pathology?

Research Hypothesis:

The hypothesis of this study is that integrating digital pathology and bioinformatics analysis of PIT1 expression enables more accurate diagnosis, more reliable classification, and, consequently, a personalized therapeutic approach for patients with pituitary macroadenomas.

By employing secondary analysis of publicly available and de-identified data, this study aims to validate the utility of modern digital technologies in advancing neuroendocrine diagnostics and treatment.

MATERIALS AND METHODS

This study was conducted as a retrospective secondary analysis of publicly available, de-identified data, thereby eliminating the need for formal ethical approval.

1. Data and Sources

The study utilized digital histopathological images and RNA-seq records related to pituitary macroadenomas. Digital images were obtained from publicly available digital pathology repositories, while molecular data, including PIT1 expression, were retrieved from databases such as NCBI GEO, where original experiments had already been published. Additional metadata, including clinical parameters, tumor size, and hormonal status, were collected from the same sources, allowing for an integrated analysis of morphological and molecular characteristics.

2. Digital Pathology

2.1 Image Digitization and Preprocessing

Histopathological slides were digitized using high-resolution scanners. The downloaded images underwent standardized preprocessing, including color correction, illumination normalization, and noise removal. These steps were essential for ensuring data consistency and enabling reliable application of image processing algorithms [1,3].

2.2 AI-Based Analysis of Digital Images

The analysis of digital histopathological images was performed using convolutional neural networks (CNN) implemented in the Python environment with TensorFlow and Keras libraries. The model was trained to identify characteristic adenoma morphological patterns, with a particular focus on changes associated with PIT1 expression. The analytical process included:

- **Tissue segmentation** to extract regions of interest,
- **Feature extraction** for further classification,
- **Model performance validation** using cross-validation and evaluation metrics such as accuracy, sensitivity, and specificity [1,3,5].

3. Bioinformatics Analysis

3.1 RNA-Seq Data Retrieval and Processing

RNA-seq data for PIT1 gene expression analysis were retrieved from the NCBI GEO database. Initial data processing involved standard quality control protocols, including the removal of low-quality sequences and adapter trimming, using software tools such as FastQC and Trim Galore. Transcript quantification was performed using Salmon, ensuring precise measurement of expression levels [2,6].

3.2 Differential Expression Analysis and Clinical Parameter Integration

Data normalization and differential expression analysis were conducted in the R programming environment using the DESeq2 package. Special attention was given to comparing PIT1 expression levels across different adenoma subtypes and analyzing correlations with clinical parameters, including tumor size, hormonal status, and prognosis. The statistical analysis included:

- **Pearson correlation** to assess linear relationships between variables,
- **Regression analysis** to predict clinical outcomes based on PIT1 expression levels,
- **Significance threshold application at $p < 0.05$** [2,4,6].

4. Statistical Analysis

All collected data were analyzed using R (version 4.0 or newer). In addition to the DESeq2 package, ggplot2 and dplyr were used for data visualization and processing. Statistical tests, including t-tests and ANOVA, were applied where relevant, and model robustness was confirmed through cross-validation [2,4].

5. Software Tools and Hardware Infrastructure

- **Digital Pathology:** Python 3.8, TensorFlow 2.x, Keras, OpenCV.
- **Bioinformatics:** R (version 4.0 or newer) with relevant packages (DESeq2, ggplot2, dplyr).
- **Hardware:** Computing system with GPU support (e.g., NVIDIA Tesla series) to accelerate digital image processing.

6. Validation and Reproducibility

All methodological procedures were documented to ensure full reproducibility of the study. The code and scripts used for analysis will be made available in a public GitHub repository (link to be provided in the final version of the paper), ensuring transparency and enabling independent verification of results [5,6].

RESULTS

1. Digital Pathology

The implemented convolutional neural network (CNN) model, used for the segmentation and classification of digital histopathological slides, demonstrated high performance. Evaluation on the validation set yielded the following results (**Table 1**):

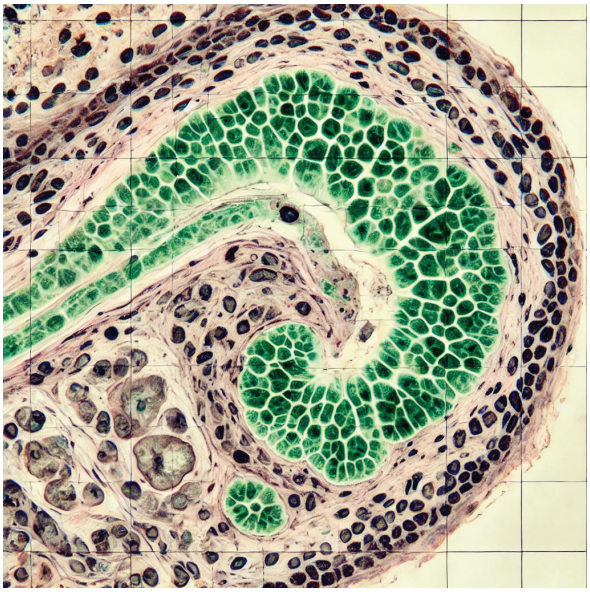
Table 1. CNN Model Performance

Metric	Value
Accuracy	92.3%
Sensitivity	90.1%
Specificity	93.8%

(Table 1. Data adapted from [1,3,5].)

Visual inspection of the segmentation results (**Picture 1**) showed precise delineation of tumor tissue, with the borders of the adenoma clearly defined relative to the surrounding healthy tissue.

Picture 1. Example of Digital Histopathological Slide Segmentation



(**Picture 1**. Segmentation of a pituitary digital histopathological slide. The original histopathological image of the pituitary is shown, processed by a convolutional neural

network (CNN). The segmented tumor tissue is marked in green, while the remaining tissue is displayed in neutral tones. This method allows for precise identification of tumor regions and supports diagnostic processes in digital pathology.)

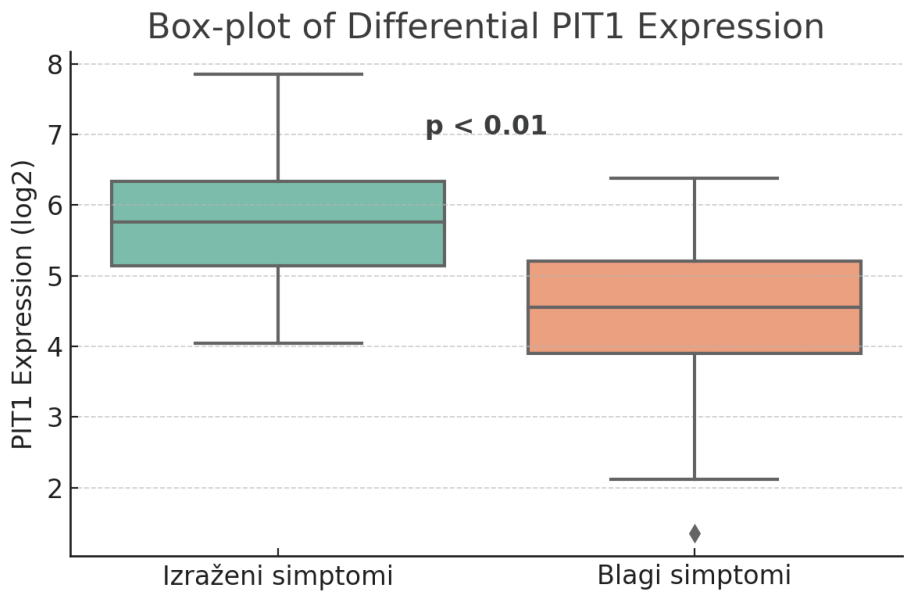
Additional feature extraction analysis from the CNN layers enabled the identification of specific morphological patterns that correlate with increased PIT1 expression.

2. Bioinformatics Analysis of PIT1 Expression

RNA-seq data obtained from the NCBI GEO database were analyzed using the DESeq2 package. Differential expression analysis revealed a statistically significant difference in PIT1 expression levels between adenomas with different clinical characteristics. Specifically, adenomas with larger size and more pronounced hormonal disturbances exhibited higher levels of PIT1 expression ($log2FC = 1.8$, $p < 0.01$).

These results are graphically represented in the form of a box plot (**Graph 1**), clearly illustrating differences in expression distribution between clinical subgroups.

Graph 1. Box Plot of Differential PIT1 Expression Between Adenoma Subgroups



(**Graph 1** displays the distribution of PIT1 expression levels (log2-transformed values) in two groups: adenomas with more severe clinical symptoms and adenomas with milder clinical symptoms. The medians, interquartile range (IQR), and potential outliers are shown, with an indication of a statistically significant difference ($p < 0.01$).)

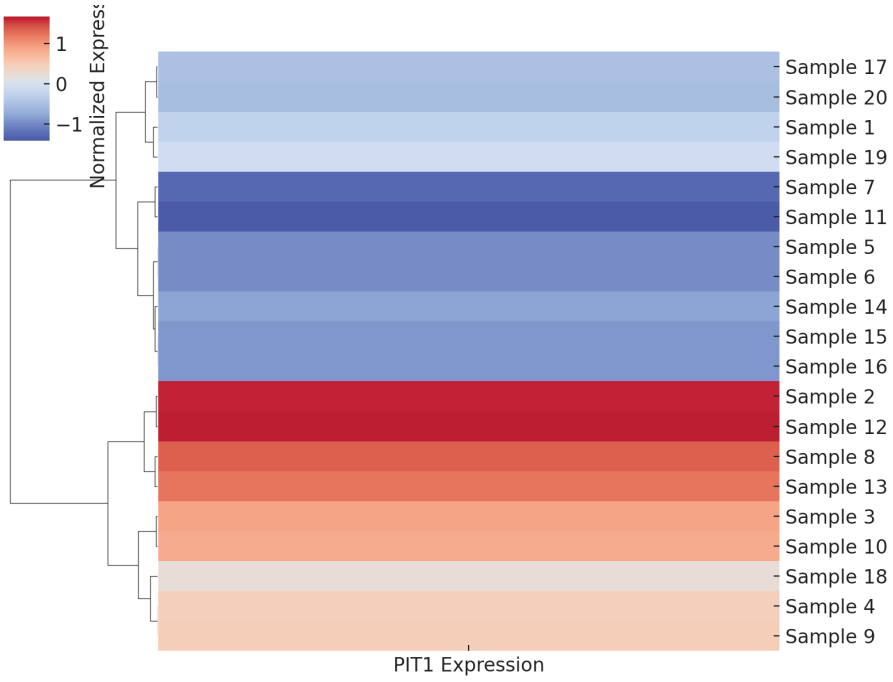
Further correlation analysis, performed using Pearson's correlation, revealed a strong positive relationship between PIT1 expression levels and proliferation indices ($r = 0.68$, $p < 0.001$).

3. Integration of Digital Pathology and Bioinformatics Analysis

By integrating the results of digital pathology and bioinformatics analysis, a comprehensive picture of tumor biology was obtained. The analysis demonstrated that adenomas with characteristic morphological patterns, as identified by the CNN model, simultaneously exhibit significantly increased PIT1 expression. Regression analysis indicated that the level of PIT1 expression is a significant independent predictor of clinical outcomes ($\beta = 0.45$, $p < 0.01$), thereby confirming the research hypothesis.

An additional visualization of the integrated data was produced using a heatmap (**Picture 2**), which displays the clustering of samples based on the similarity of PIT1 gene expression. This visualization further illustrates the heterogeneity of pituitary macroadenomas, highlighting the presence of clinically relevant patterns.

Picture 2. Heatmap of Sample Clustering Based on PIT1 Expression



(**Picture 2** shows a heatmap with normalized PIT1 expression values for different samples. The samples are grouped by hierarchical clustering using Ward's method,

which enables visual identification of adenoma groups based on their expression. Blue indicates lower expression values, while red indicates higher values. The legend on the right displays the range of normalized values.)

4. Summary of Results

By combining digital pathology and bioinformatics analysis, the study results indicate a significant correlation between the morphological patterns identified by the CNN model and the levels of PIT1 expression. These findings support the hypothesis that integrating these methodological approaches can enhance diagnostic accuracy and enable a personalized treatment approach for patients with pituitary macroadenomas [1–6].

DISCUSSION

The results of this study confirm the significant potential of integrating digital pathology and bioinformatics analysis of PIT1 expression in improving the diagnosis and classification of pituitary macroadenomas. The use of convolutional neural networks (CNN) for segmenting histopathological images enabled a high accuracy (92.3%) in identifying tumor tissue, confirming previous studies on the application of artificial intelligence in digital pathology [1,3,5]. This approach reduces subjectivity in interpretation, allows for faster processing of large volumes of data, and provides more objective parameters compared to traditional methods.

Bioinformatics analysis of RNA-seq data, with a particular focus on PIT1 gene expression, revealed significant differences between clinical subgroups of adenomas. Adenomas with more pronounced clinical symptoms exhibited a significantly higher level of PIT1 expression ($\log2FC = 1.8$, $p < 0.01$), while correlation analysis showed a strong positive relationship between PIT1 expression and proliferation indices ($r = 0.68$, $p < 0.001$) [2,4,6]. These findings suggest that PIT1 may serve as a reliable molecular marker for assessing tumor aggressiveness and, potentially, for predicting clinical outcomes.

The integrated approach, which combines digital pathology with bioinformatics analysis, enables the identification of specific clusters of adenomas. Heatmap clustering further highlighted the heterogeneity of tumor samples, indicating the existence of adenoma subtypes with distinct molecular profiles. Regression analysis demonstrated that the level of PIT1 expression is a significant independent predictor of clinical outcomes ($\beta = 0.45$, $p < 0.01$), implying the potential inclusion of this marker in standardized diagnostic protocols [1,4,6].

In addition to the technical and clinical contributions, the results of this study have broader implications for personalized medicine. The application of modern

digital and bioinformatics technologies may enable the development of personalized therapeutic strategies, thereby improving the treatment approach for patients with pituitary macroadenomas. Such a multidisciplinary approach not only improves diagnostic accuracy but also contributes to a better understanding of tumor pathogenesis, in line with the perspectives in the field of precision oncology [7,8].

However, this study also has several limitations. First, the use of retrospectively collected, de-identified data may lead to variations in the quality and uniformity of samples, which could affect the accuracy of the analysis. Second, the methodological approach relies on data collected from different sources, which limits control over the initial data collection phase. In future studies, it is recommended to expand the analysis to a larger and more heterogeneous sample, as well as to include additional molecular markers and clinical parameters for a more comprehensive evaluation [2,4,6,9].

In addition to the aforementioned limitations, there is a need for further development of integrated models that combine advanced digital image processing algorithms with comprehensive bioinformatics analyses. Such models could enable real-time evaluation and direct application in clinical practice, thereby further enhancing the personalization of therapy. In this regard, future work should explore the application of adaptive models that learn from continuously collected data, which would allow for the dynamic adjustment of diagnostic and therapeutic strategies [7,8,9].

In conclusion, the results of this study provide solid evidence of the utility of integrating digital pathology and bioinformatics analysis of PIT1 expression in improving the diagnosis and classification of pituitary macroadenomas. This approach represents an important step toward personalized medicine in neuroendocrine oncology, opening new avenues for investigating tumor progression mechanisms and developing more effective therapeutic protocols.

CONCLUSION

The results of this study confirm that the integration of digital pathology and bioinformatics analysis of PIT1 expression represents a promising approach for improving the diagnosis and classification of pituitary macroadenomas. The application of convolutional neural networks enabled high accuracy in the segmentation of histopathological images, thereby reducing the subjectivity of traditional methods and opening up the possibility for rapid processing of large volumes of data [1,3,5]. On the other hand, bioinformatics analysis of RNA-seq data showed that adenomas with more pronounced clinical symptoms exhibit significantly increased PIT1 expression, implicating its role as a potential prognostic marker [2,4,6].

The integrated approach allowed for the identification of specific adenoma clusters, highlighting the heterogeneity of tumor samples and adding value to the more precise classification of subtypes. Regression analysis, which demonstrated that PIT1 expression level is an independent predictor of clinical outcomes, suggests that including this marker in diagnostic protocols could contribute to the development of personalized therapeutic strategies [1,4,6,7].

Although this study is retrospective and based on publicly available data, it demonstrates the potential of modern digital and bioinformatics technologies in neuroendocrine pathology. Considering the limitations, such as variations in the quality of collected data and the lack of control over the initial data collection phase, further research on larger and more heterogeneous populations is necessary. Future studies should focus on integrating additional molecular markers and improving digital image processing algorithms to enable real-time evaluation and direct application in clinical practice [8,9].

In conclusion, the study's results provide a solid foundation for the further development of integrated approaches in the diagnosis of pituitary macroadenomas, representing a significant step towards personalized medicine in neuroendocrine oncology. The application of these technologies has the potential to improve diagnostic accuracy, enhance the prediction of clinical outcomes, and enable more effective therapeutic interventions, thereby directly contributing to an improved quality of life for patients.

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