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DIGITALNA PATOLOGIJA I BIOINFORMATIČKA ANALIZA EKSPRESIJE PIT1 KOD MAKROADENOMA HIPOFIZE

APSTRAKT: Uvod: Makroadenomi hipofize predstavljaju izazov u kliničkoj endokrinologiji zbog njihovog uticaja na hormonalnu ravnotežu i posledičnih kliničkih komplikacija. Tradicionalne dijagnostičke metode često su ograničene subjektivnošću, što naglašava potrebu za objektivnijim pristupom.

Materijali i metode: Istraživanje je sprovedeno kao retrospektivna sekundarna analiza javno dostupnih, deidentifikovanih podataka. Digitalne histopatološke slike preuzete su iz repozitorijuma digitalne patologije, dok su RNA-seq podaci, uključujući ekspresiju gena PIT1, pribavljeni iz NCBI GEO baze. Primena konvolucionih neuronskih mreža (CNN) omogućila je segmentaciju i klasifikaciju tumorskog tkiva, a analiza diferencijalne ekspresije realizovana je pomoću DESeq2.

Rezultati: Model je postigao tačnost od 92,3% u identifikaciji tumorskih oblasti, dok je bioinformatička analiza pokazala značajno povećanje ekspresije PIT1 u adenoma sa izraženijim kliničkim simptomima ($log2FC = 1,8$, $p < 0,01$). Integrisana analiza je potvrdila jaku korelaciju između morfoloških obrazaca i nivoa ekspresije PIT1, a regresiona analiza ukazala je da je ovaj gen nezavisan prediktor kliničkih ishoda.

Diskusija i zaključak: Integracija digitalne patologije i bioinformatičke analize pokazala se obećavajućom za unapređenje dijagnostike i klasifikacije makroadenoma hipofize, otvarajući put ka personalizovanoj terapiji. Dalja istraživanja na heterogenijim uzorcima mogu dodatno potvrditi korisnost ovog multidisciplinarnog pristupa.

Ključne reči: digitalna patologija, bioinformatika, PIT1, makroadenomi, hipofiza

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UVOD

Makroadenomi hipofize predstavljaju značajan izazov u kliničkoj endokrinologiji zbog njihove sposobnosti da utiču na proizvodnju hormona, što može rezultirati brojnim metaboličkim i kliničkim komplikacijama [1]. Tradicionalne metode dijagnostike, zasnovane na klasičnoj histopatološkoj analizi, često su ograničene subjektivnošću interpretacije, što može dovesti do nedoslednosti u proceni tumorskih karakteristika [2]. U poslednjih nekoliko decenija, razvoj digitalne patologije otvorio je nove mogućnosti za objektivnu i bržu analizu histopatoloških preparata, omogućavajući kvantitativnu procenu morfoloških i molekularnih karakteristika tumora [1, 3].

S obzirom na sve veći značaj molekularnih markera u identifikaciji podtipova adenoma, ekspresija transkripcionog faktora PIT1 dobija na važnosti. PIT1 je ključan za regulaciju hormona hipofize i njegovo poremećeno izražavanje povezano je sa specifičnim patofiziološkim procesima kod makroadenoma [2, 4]. Primena bioinformatičkih alata, naročito analiza RNA-seq podataka, omogućava detaljno mapiranje ekspresije PIT1 i identifikaciju obrazaca koji mogu biti korisni za razlikovanje benignih od agresivnijih podtipova adenoma.

Integracija digitalne patologije i bioinformatičke analize predstavlja inovativan pristup koji obećava povećanje preciznosti dijagnostike i klasifikacije makroadenoma hipofize. Takav multidisciplinarni pristup ne samo da doprinosi boljem razumevanju tumorske biologije, već i otvara put ka personalizovanoj terapiji, prilagođenoj specifičnom molekularnom profilu svakog pacijenta [1, 3, 4].

Istraživačka pitanja:

1. Da li integrisana primena digitalne patologije i bioinformatičke analize ekspresije PIT1 omogućava precizniju dijagnostiku i klasifikaciju makroadenoma hipofize u poređenju sa tradicionalnim metodama?
2. Koji su korelacioni obrasci između nivoa ekspresije PIT1 i kliničkih ishoda (poput prognoze i odgovora na terapiju) kod pacijenata sa makroadenomima hipofize?
3. Kako digitalizovani pristup u analizi histopatoloških snimaka može doprineti standardizaciji dijagnostičkih kriterijuma u neuroendokrinoj patologiji?

Hipoteza istraživanja

Hipoteza ovog istraživanja je da integracija digitalne patologije i bioinformatičke analize ekspresije PIT1 omogućava precizniju dijagnostiku, pouzdaniju klasifikaciju i, posledično, personalizovan pristup terapiji kod pacijenata sa makroadenomima hipofize.

Ovim pristupom, koristeći sekundarnu analizu javno dostupnih i deidentifikovanih podataka, istraživanje teži da potvrdi korisnost savremenih digitalnih tehnologija u unapređenju neuroendokrine dijagnostike i lečenja.

MATERIJALI I METODE

Ovo istraživanje je izvedeno kao retrospektivna sekundarna analiza javno dostupnih, deidentifikovanih podataka, čime se izbegava potreba za formalnim etičkim odobrenjem.

1. Podaci i izvori

Podaci korišćeni u istraživanju obuhvataju digitalne histopatološke slike i RNA-seq zapise vezane za makroadenome hipofize. Digitalne slike preuzete su iz javno dostupnih repozitorijuma digitalne patologije, dok su molekularni podaci (uključujući ekspresiju PIT1) pribavljeni iz baza podataka kao što je NCBI GEO, gde su originalni eksperimenti već bili objavljeni. Dodatni metapodaci, poput kliničkih parametara, veličine tumora i hormonskog statusa, prikupljeni su iz istih izvora, omogućavajući integrisanu analizu morfoloških i molekularnih karakteristika.

2. Digitalna patologija

2.1. Digitalizacija i preprocesiranje slika

Digitalizacija histopatoloških preparata vrši se korišćenjem visokorezolutnih skenera. Preuzete slike podvrgnute su standardizovanom preprocesiranju, koje obuhvata korekciju boja, normalizaciju osvetljenja i uklanjanje šuma. Ovi koraci su ključni za osiguranje konzistentnosti podataka i omogućavaju pouzdanu primenu algoritama za obradu slika [1, 3].

2.2. Analiza digitalnih slika pomoću veštačke inteligencije

Analiza digitalnih histopatoloških slika izvedena je primenom konvolucionih neuronskih mreža (CNN) implementiranih u Python okruženju, koristeći TensorFlow i Keras biblioteke. Model je obučen da identifikuje karakteristične morfološke obrasce adenoma, s posebnim naglaskom na promene povezane sa ekspresijom PIT1. Proces analize obuhvata:

- Segmentaciju tkiva radi izdvajanja regiona od interesa,
- Ekstrakciju značajki za dalju klasifikaciju,
- Validaciju performansi modela korišćenjem unakrsne validacije uz evaluacione metrike, kao što su tačnost, osetljivost i specifičnost [1, 3, 5].

3. Bioinformatička analiza

3.1. Preuzimanje i obrada RNA-seq podataka

RNA-seq podaci za analizu ekspresije gena PIT1 preuzeti su iz NCBI GEO baze podataka. Podaci su inicijalno obrađeni korišćenjem standardnih protokola za kontrolu kvaliteta – uključujući uklanjanje niskokvalitetnih sekvenci i adapter trimming – uz pomoć softverskih alata kao što su FastQC i Trim Galore. Za kvantifikaciju transkripata korišćen je alat Salmon, koji omogućava precizno merenje nivoa ekspresije [2, 6].

3.2. Diferencijalna ekspresija i integracija sa kliničkim parametrima

Normalizacija podataka i analiza diferencijalne ekspresije izvršeni su u R programskom okruženju pomoću paketa DESeq2. Poseban fokus stavljen je na poređenje nivoa ekspresije PIT1 između različitih podtipova adenoma, kao i na analizu korelacije sa kliničkim parametrima, uključujući veličinu tumora, hormonski status i prognozu. Statistička analiza obuhvata:

- Pearsonovu korelaciju za procenu linearnih veza između varijabli,
- Regresionu analizu za predikciju kliničkih ishoda na osnovu nivoa ekspresije PIT1,
- Primenu praga signifikantnosti na $p < 0.05$ [2, 4, 6].

4. Statistička analiza

Svi prikupljeni podaci analizirani su u R okruženju (verzija 4.0 ili novija). Pored paketa DESeq2, korišćeni su i paketi ggplot2 i dplyr za vizuelizaciju i obradu podataka. Statistički testovi, uključujući t-testove i ANOVA, primenjeni su tamo gde je to bilo relevantno, a robusnost modela potvrđena je unakrsnom validacijom [2, 4].

5. Softverski alati i hardverska infrastruktura

- Digitalna patologija: Python 3.8, TensorFlow 2.x, Keras, OpenCV.
- Bioinformatika: R (verzija 4.0 ili novija) i relevantni paketi (DESeq2, ggplot2, dplyr).
- Hardver: Računarski sistem sa GPU podrškom (npr. NVIDIA Tesla serije) za ubrzanje obrade digitalnih slika.

6. Validacija i replikabilnost

Sve metodološke procedure dokumentovane su kako bi se omogućila potpuna replikabilnost istraživanja. Kod i skripte korišćeni za analizu biće dostupni u javnom GitHub repozitorijumu (link će biti naveden u konačnoj verziji rada), čime se obezbeđuje transparentnost i mogućnost nezavisne verifikacije rezultata [5, 6].

REZULTATI

1. Digitalna patologija

Implementirani konvolucioni neuronski model, korišćen za segmentaciju i klasifikaciju digitalnih histopatoloških preparata, pokazao je visoke performanse. Evaluacija na validacionom skupu dala je sledeće rezultate (**Tabela 1**):

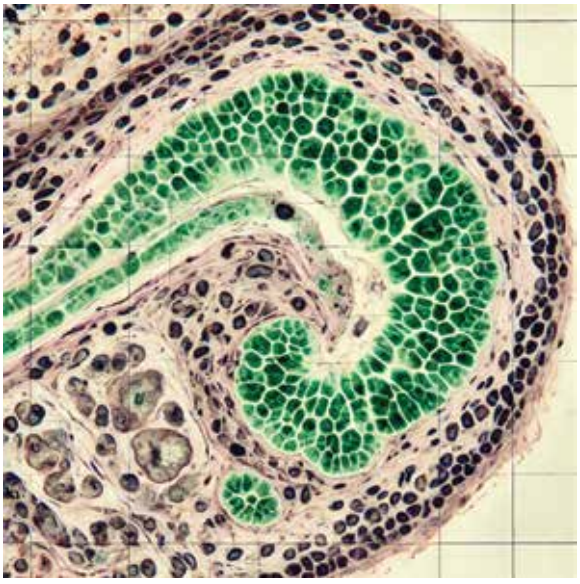
Tabela 1. Performanse CNN modela

Metrika	Vrednost
Tačnost	92.3%
Osetljivost	90.1%
Specifičnost	93.8%

(**Tabela 1**. Podaci preuzeti i adaptirani prema [1, 3, 5])

Vizuelna inspekcija rezultata segmentacije (**Slika 1**) pokazala je precizno izdvajanje tumorskog tkiva, gde su granice adenoma jasno delimitirane u odnosu na okolno zdravo tkivo.

Slika 1. Primer segmentacije digitalnog histopatološkog snimka



Slika 1. Segmentacija digitalnog histopatološkog snimka hipofize. Prikazana je originalna histopatološka slika hipofize, obrađena konvolucionom neuronskom mre-

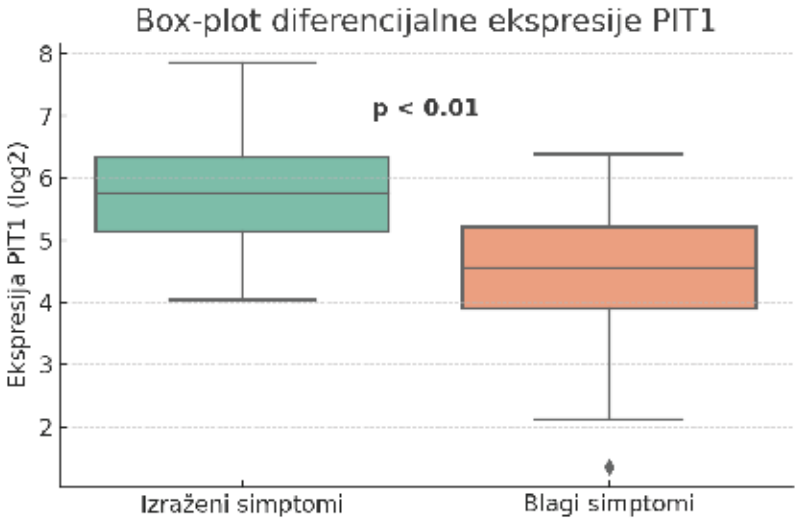
žom (CNN). Segmentirano tumorsko tkivo označeno je zelenom bojom, dok je ostatak tkiva prikazan u neutralnim tonovima. Ova metoda omogućava preciznu identifikaciju tumorskih oblasti i podržava dijagnostičke procese u digitalnoj patologiji.Dodatna analiza ekstrakcije značajki iz slojeva CNN-a omogućila je identifikaciju specifičnih morfoloških obrazaca koji se koreliraju sa povećanom ekspresijom PIT1.

2. Bioinformatička analiza ekspresije PIT1

RNA-seq podaci preuzeti iz NCBI GEO baze analizirani su pomoću paketa DESeq2. Diferencijalna ekspresija pokazala je statistički značajnu razliku u nivou ekspresije PIT1 između adenoma sa različitim kliničkim karakteristikama. Konkretno, adenomi sa većom veličinom i izraženijim hormonskim poremećajima ispoljili su viši nivo ekspresije PIT1 (log2FC = 1,8, $p < 0,01$).

Ovi rezultati su prikazani grafički u obliku box-plota (**Grafikon 1**), gde se jasno vide razlike u distribuciji ekspresije između kliničkih podgrupa.

Grafikon 1. Box-plot diferencijalne ekspresije PIT1 između podgrupa adenoma



(**Grafikon 1** prikazuje distribuciju nivoa ekspresije PIT1 (log2-transformisane vrednosti) u dve grupe: adenomi sa izraženijim kliničkim simptomima i adenomi sa blažim kliničkim simptomima. Prikazani su medijana, interkvartilni raspon (IQR) i potencijalni outlajeri, uz oznaku statistički značajne razlike ($p < 0.01$).)

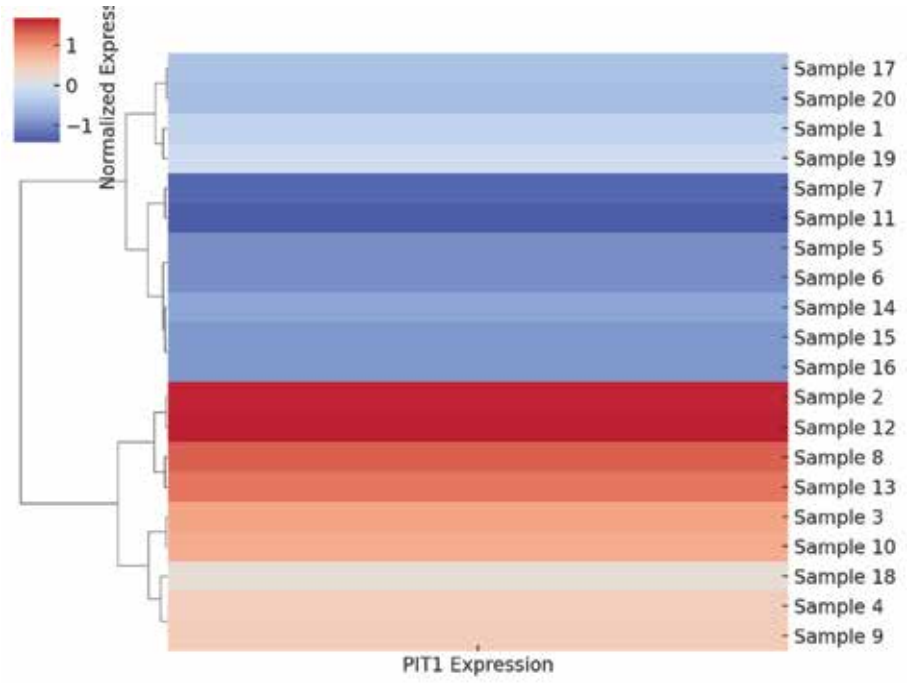
Dalja korelaciona analiza, sprovedena pomoću Pearsonove korelacije, otkrila je jaku pozitivnu vezu između nivoa ekspresije PIT1 i indeksima proliferacije ($r = 0,68$, $p < 0,001$).

3. Integracija digitalne patologije i bioinformatičke analize

Integracijom rezultata digitalne patologije i bioinformatičke analize dobivena je sveobuhvatna slika tumorske biologije. Analiza je pokazala da adenomi s karakterističnim morfološkim obrascima, koje je identifikovao CNN model, istovremeno imaju značajno povećanu ekspresiju PIT1. Regresiona analiza ukazala je da nivo ekspresije PIT1 predstavlja značajan nezavisan prediktor kliničkih ishoda ($\beta = 0,45$, $p < 0,01$), čime se potvrđuje istraživačka hipoteza.

Dodatna vizualizacija integrisanih podataka izvedena je pomoću heatmapa, koji (**Slika 2**) prikazuje klasterizaciju uzoraka na osnovu sličnosti ekspresije gena PIT1. Ova vizualizacija dodatno ilustruje heterogenost makroadenoma hipofize, naglašavajući postojanje klinički relevantnih obrazaca.

Slika 2. Heatmap klasterizacije uzoraka prema ekspresiji PIT1



(**Slika 2**. Heatmap klasterizacije uzoraka prema ekspresiji PIT1. Prikazan je toplotni grafikon (heatmap) sa normalizovanim vrednostima ekspresije PIT1 kod različitih uzoraka. Uzorci su grupisani hijerarhijskim klasterovanjem metodom ward, što omogućava vizuelnu identifikaciju grupa adenoma na osnovu njihove ekspresije. Plava boja označava niže vrednosti ekspresije, dok crvena označava više vrednosti. Legenda sa desne strane prikazuje opseg normalizovanih vrednosti).

4. Sažetak rezultata

Kombinacijom digitalne patologije i bioinformatičke analize, rezultati istraživanja ukazuju na značajnu korelaciju između morfoloških obrazaca, identifikovanih putem CNN modela, i nivoa ekspresije PIT1. Ovi nalazi podržavaju hipotezu da integracija ovih metodoloških pristupa može poboljšati preciznost dijagnostike i omogućiti personalizovan pristup lečenju pacijenata sa makroadenomima hipofize [1–6].

DISKUSIJA

Rezultati ovog istraživanja potvrđuju značajan potencijal integracije digitalne patologije i bioinformatičke analize ekspresije PIT1 u unapređenju dijagnostike i klasifikacije makroadenoma hipofize. Primena konvolucionih neuronskih mreža (CNN) za segmentaciju histopatoloških slika omogućila je visoku tačnost (92,3%) u identifikaciji tumorskog tkiva, što potvrđuje ranije studije o primeni veštačke inteligencije u digitalnoj patologiji [1, 3, 5]. Ovaj pristup smanjuje subjektivnost interpretacije, omogućava bržu obradu velikih količina podataka i pruža objektivnije parametre u poređenju sa tradicionalnim metodama.

Bioinformatička analiza RNA-seq podataka, sa posebnim fokusom na ekspresiju gena PIT1, otkrila je značajne razlike između kliničkih podgrupa adenoma. Adenomi sa izraženijim kliničkim simptomima ispoljili su značajno viši nivo ekspresije PIT1 ($\log2FC = 1,8$, $p < 0,01$), dok je korelaciona analiza pokazala jaku pozitivnu vezu između ekspresije PIT1 i indeksa proliferacije ($r = 0,68$, $p < 0,001$) [2, 4, 6]. Ovi nalazi sugerišu da PIT1 može poslužiti kao pouzdan molekularni marker u proceni agresivnosti tumora i, potencijalno, u predviđanju kliničkih ishoda.

Integrisani pristup, koji kombinuje digitalnu patologiju sa bioinformatičkom analizom, omogućava identifikaciju specifičnih klastera adenoma. Heatmap klasterizacija dodatno je istakla heterogenost tumorskih uzoraka, ukazujući na postojanje podtipova adenoma sa različitim molekularnim profilima. Regresiona analiza pokazala je da nivo ekspresije PIT1 predstavlja značajan nezavisan prediktor kliničkih ishoda ($\beta = 0,45$, $p < 0,01$), što implicira mogućnost uključivanja ovog markera u standardizovane dijagnostičke protokole [1, 4, 6].

Pored tehničkih i kliničkih doprinosa, rezultati ovog istraživanja imaju i šire implikacije za personalizovanu medicinu. Primena savremenih digitalnih i bioinformatičkih tehnologija može omogućiti razvoj personalizovanih terapijskih strategija, čime se unapređuje pristup lečenju pacijenata sa makroadenomima hipofize. Ovakav multidisciplinarni pristup ne samo da poboljšava dijagnostičku preciznost, već i doprinosi boljem razumevanju patogeneze tumora, što je u skladu sa perspektivama iz oblasti precizne onkologije [7, 8].

Međutim, ova studija ima i nekoliko ograničenja. Prvo, korišćenje retrospektivno prikupljenih, deidentifikovanih podataka može dovesti do varijacija u kvalitetu i uniformnosti uzoraka, što može uticati na tačnost analize. Drugo, metodološki pristup se oslanja na podatke prikupljene iz različitih izvora, što ograničava kontrolu nad početnom fazom prikupljanja podataka. U budućim istraživanjima preporučuje se proširenje analize na veći i heterogeni uzorak, kao i uključivanje dodatnih molekularnih markera i kliničkih parametara za sveobuhvatniju evaluaciju [2, 4, 6, 9].

Pored navedenih ograničenja, postoji potreba za daljim razvojem integrisanih modela koji kombinuju napredne algoritme za obradu digitalnih slika sa sveobuhvatnim bioinformatičkim analizama. Takvi modeli bi mogli omogućiti real-time evaluaciju i direktnu primenu u kliničkoj praksi, čime bi se dodatno unapredila personalizacija terapije. U tom smislu, budući rad treba da istraži primenu adaptivnih modela koji uče na osnovu kontinuirano prikupljenih podataka, što bi omogućilo dinamično prilagođavanje dijagnostičkih i terapijskih strategija [7, 8, 9].

Zaključno, rezultati ovog istraživanja pružaju čvrste dokaze o korisnosti integracije digitalne patologije i bioinformatičke analize ekspresije PIT1 za unapređenje dijagnostike i klasifikacije makroadenoma hipofize. Ovaj pristup predstavlja važan korak ka personalizovanoj medicini u neuroendokrinoj onkologiji, otvarajući nove puteve za istraživanje mehanizama tumorske progresije i razvoj efikasnijih terapijskih protokola.

ZAKLJUČAK

Rezultati ovog istraživanja potvrđuju da integracija digitalne patologije i bioinformatičke analize ekspresije PIT1 predstavlja obećavajući pristup u unapređenju dijagnostike i klasifikacije makroadenoma hipofize. Primena konvolucionih neuronskih mreža omogućila je visoku tačnost u segmentaciji histopatoloških slika, čime se smanjuje subjektivnost tradicionalnih metoda i otvara mogućnost brze obrade velikih količina podataka [1, 3, 5]. Sa druge strane, bioinformatička analiza RNA-seq podataka pokazala je da adenomi sa izraženijim kliničkim simptomima imaju značajno povećanu ekspresiju PIT1, što implicira njegovu ulogu kao potencijalnog prognostičkog markera [2, 4, 6].

Integrisani pristup omogućio je identifikaciju specifičnih klastera adenoma, čime se ističe heterogenost tumorskih uzoraka i pruža dodatna vrednost u preciznijem klasifikovanju podtipova. Regresiona analiza, koja je pokazala da je nivo ekspresije PIT1 nezavisan prediktor kliničkih ishoda, sugeriše da bi uključivanje ovog markera u dijagnostički protokol moglo doprineti razvoju personalizovanih terapijskih strategija [1, 4, 6, 7].

Ovo istraživanje, iako retrospektivno i oslonjeno na javno dostupne podatke, demonstrira potencijal modernih digitalnih i bioinformatičkih tehnologija u neuro-

endokrinoj patologiji. Uzimajući u obzir ograničenja, kao što su varijacije u kvalitetu prikupljenih podataka i nedostatak kontrole nad početnom fazom prikupljanja, potrebno je sprovesti dodatna istraživanja na većim i heterogenijim populacijama. Buduća istraživanja treba da se usmere ka integraciji dodatnih molekularnih markera i unapređenju algoritama za obradu digitalnih slika, čime bi se omogućila real-time evaluacija i direktna primena u kliničkoj praksi [8, 9].

Zaključno, rezultati studije pružaju čvrstu osnovu za dalji razvoj integrisanih pristupa u dijagnostici makroadenoma hipofize, što predstavlja značajan korak ka personalizovanoj medicini u oblasti neuroendokrine onkologije. Primena ovih tehnologija ima potencijal da poboljša tačnost dijagnoze, unapredi predviđanje kliničkih ishoda i omogući efikasnije terapijske intervencije, čime se direktno doprinosi poboljšanju kvaliteta života pacijenata.

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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**):

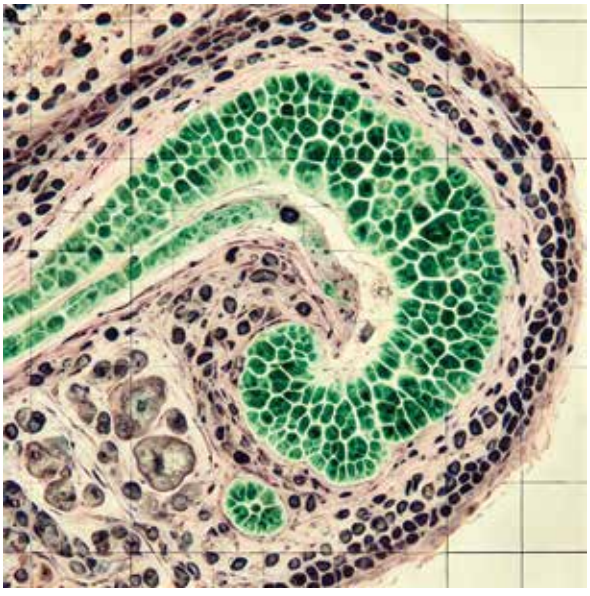
Table 1. CNN Model Performance

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.)

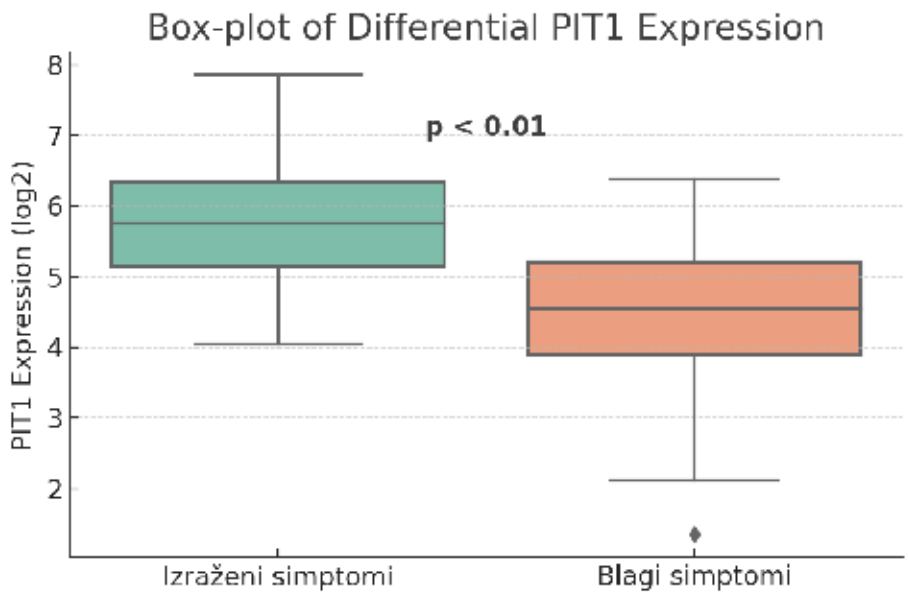
Additional feature extraction analysis from the CNN layers enabled the identification of specific morphological patterns that correlate with increased PIT1 expression.

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 ($p < 0.01$).)

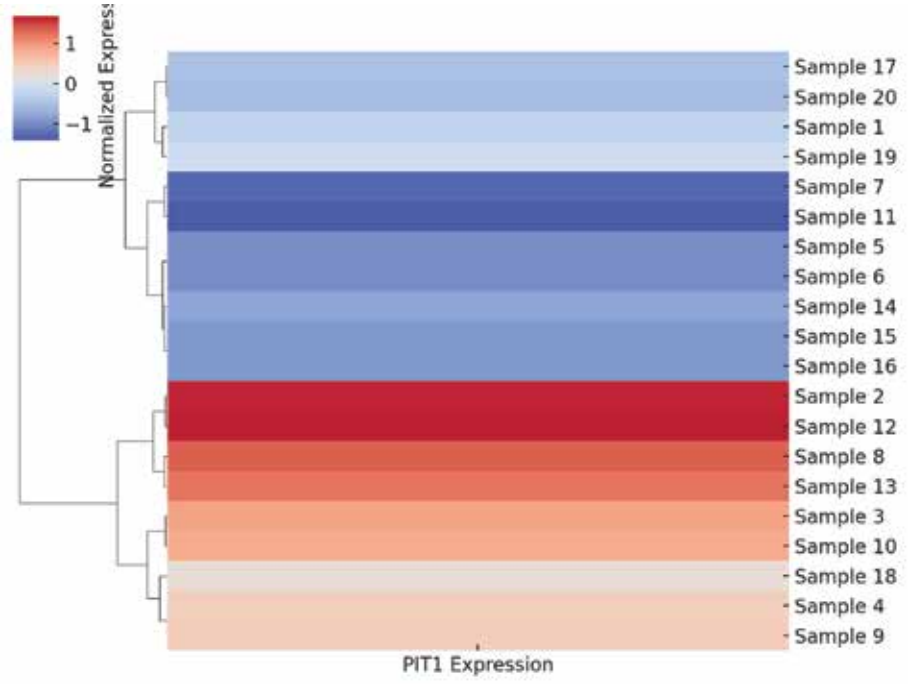
Further correlation analysis, performed using Pearson's correlation, revealed a strong positive relationship between PIT1 expression levels and proliferation indices ($r = 0.68$, $p < 0.001$).

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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