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Comprehensive Analysis of Core Genes, Key Pathways, and Immune Infiltration in Intervertebral Disc Degeneration Using Machine Learning and Experimental Validation

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ABSTRACT

This study integrated and analyzed two sets of gene expression data related to intervertebral disc degeneration (IVDD) to elucidate its key molecular mechanisms.

Through screening and enrichment analysis of differentially expressed genes (DEGs), 112 DEGs were identified, primarily involved in extracellular matrix remodeling, cytoplasmic translation, and signaling pathways such as PI3K-Akt.

A protein-protein interaction network combined with LASSO and SVM-RFE machine learning algorithms identified 13 hub genes. Immune infiltration analysis revealed reduced infiltration of suppressor cells and monocytes in IVDD samples. In an IL-1β-induced human nucleus pulposus cell degeneration model, qPCR and Western blot experiments confirmed significant downregulation of ADM, ITGB5, RTN4, SLPI, and CSNK1E expression.

This study systematically reveals the potential molecular networks and immune characteristics of IVDD, providing new candidate biomarkers and therapeutic insights for subsequent targeted drug development.

Keywords: Bioinformatics analysis; Gene differential expression; Intervertebral disc degeneration; Infiltration of immune cells; Investigation of drug-gene interactions

INTRODUCTION

Low back pain (LBP) is a prevalent musculoskeletal condition that impacts a significant proportion of the global adult population.[1] Among the several reasons, intervertebral disc degeneration (IVDD) has been continuously recognized as a major contributor to LBP.[2,3] The multifaceted development of IVDD encompasses a combination of mechanical, age-related, and inflammatory elements, as well as several biochemical changes.[4–6] Contemporary therapies for

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IVDD mostly center around pharmaceuticals and surgical procedures. However, these methods frequently only offer temporary alleviation of symptoms, without directly targeting the underlying degenerative mechanisms.[7] Hence, it is essential to delve into the fundamental processes of IVDD with more thoroughness in order to construct more efficient therapeutic interventions.

Recent studies have emphasized the vital importance of the immune system in the progressive development of IVDD.[8,9] However, the majority of these studies have focused on a limited number of immune cell types, notably macrophages. A systematic and comprehensive analysis of a broader immune cell landscape in IVDD remains underexplored. After infiltrating the intervertebral disc, macrophages undergo polarization and produce cytokines that together regulate the aging of nucleus pulposus cells (NPCs) and immune-inflammatory responses during IVDD.[10] Kämpe et al recently showed that heightened activity of IL-17–producing T_H17 cells has a role in the initiation of disc degeneration and the related low back pain in patients.[11] In a similar vein, Hanaei et al emphasized the substantial role of immunological processes, namely inflammation, in promoting cervical degeneration.[12] It is now widely acknowledged that immune infiltration plays a crucial role in the development and progression of IVDD. In a mouse model of a ruptured intervertebral disc, Lee et al reported the presence of macrophage infiltration (F4/8 ir) and increased nerve fiber density (PGP9.5-ir).[13] Furthermore, Kawakubo et al established that M1 macrophages, which are recruited after a disc damage, have a substantial impact on the inflammation that occurs after the injury.[14] Although there is increasing evidence of macrophage participation in IVDD, the role of other immune cells is not well understood. Therefore, further research is needed to investigate immunological dysregulation as a possible therapeutic target in IVDD.

MATERIALS AND METHODS

Data Resources and Preprocessing

The gene expression profiling datasets pertinent to IVDD were acquired from the Gene Expression Omnibus (GEO) database, namely GSE23130 and GSE167199, accessible at https://www.ncbi.nlm.nih.gov/geo/. The GSE23130 dataset was created utilizing the GPL1352 [U133_X3P] Affymetrix Human X3P Array platform, while the GSE167199 dataset was derived from the GPL24676 Illumina NovaSeq 6000 platform, employing *Homo sapiens*. The GSE23130 dataset comprised 8 samples of annulus disc tissue obtained from patients with Intervertebral Disc Degeneration (IVDD) and 15 samples obtained from healthy persons. The GSE167199 dataset comprised 3 samples from patients with intraventricular dysfunction (IVDD) and 3 samples from a control group with spinal cord damage. The R program "sva" was employed to merge and standardize the datasets from GSE23130 and GSE167199. The corrected data were then used for further analysis. To increase the statistical power and sample size for a more robust analysis, the gene expression profiles from datasets GSE23130 and GSE167199 were merged. The sva R package was utilized to integrate the datasets and correct for potential batch effects introduced by the different platforms (Affymetrix Human X3P Array and Illumina NovaSeq 6000) using the ComBat algorithm. This approach allows for the integration of publicly available data while minimizing technical variations, thereby enhancing the reliability of subsequent differential expression analysis.

Differential Expression Analysis

A differential expression analysis was conducted on the combined dataset using the "limma" R package.[15,16] To provide robust and statistically meaningful results, we reported the effect size for each gene as the $\log_2$ fold change ($\log_2$FC) and calculated 95% confidence intervals (CIs) for these $\log_2$FC estimates using the empirical Bayes moderation method implemented in limma. A significant criterion of an absolute $\log_2$FC>1 and a false discovery rate (FDR) adjusted p value <0.05 was applied to define differentially expressed genes (DEGs). The complete list of DEGs, including $\log_2$FC, 95% CIs, and adjusted p values, is provided in Supplementary Table S1. DEGs were represented graphically using heatmaps and volcano plots created with the "ggplot2" tool in the R programming language.[17] The significance threshold was set at a minimum overlap of 3 and a significance level of 0.01. Furthermore, the enrichment analyses were graphically represented using the "ggplot2" utility. In addition, we generated protein-protein interaction (PPI) networks using the STRING database,[18] and further visualized them in Cytoscape.[19]

Machine Learning-based Identification of Hub Genes

The LASSO regression approach provided in the "glmnet" package, together with the support vector machine (SVM) feature selection algorithm from the "e1071" package, was used to identify the key genes related to IVDD among the identified DEGs. Identified genes shared by both methods were visually represented using a Venn diagram generated with an online tool available at http://bioinformatics.psb.ugent.be/webtools/Venn/. The least absolute shrinkage and selection operator (LASSO) regression analysis was performed using the glmnet package with 10-fold cross-validation to identify the optimal penalty parameter (lambda). Similarly, the SVM-recursive feature elimination (SVM-RFE) algorithm was implemented using the e1071 package, also with 10-fold cross-validation, to rank the importance of the DEGs.

Quantification of Immune Infiltration

RNA-seq data from patients with Intervertebral Disc Degeneration (IVDD) and healthy controls were examined using single-sample gene-set enrichment analysis (ssGSEA) to determine the relative numbers of 28 different types of immune cells. Immune cell infiltration in each sample was evaluated using enrichment scores obtained from ssGSEA. Variations in immune cell expression were then shown using the "ggplot2" software. Significant correlations between immune cell expression and hub genes were shown in a heatmap, with a predetermined statistical significance level of $p<0.05$.

Analysis of mRNA-TF and mRNA-drug Interaction Networks

To elucidate the regulatory and therapeutic landscape of the hub genes, we constructed interaction networks with transcription factors (TFs) and drugs. Potential TFs were identified using the MotifMap database. Putative therapeutic agents targeting the hub genes were retrieved from the Drug-Gene Interaction Database (DGIdb). To ensure the reliability of the drug-gene interactions, we applied stringent filters within DGIdb: only interactions with a known mechanism of action and supported by at least one curated source (DrugBank) were selected. Furthermore, an interaction score threshold of >0.05 was applied to prioritize high-confidence predictions. The resulting mRNA-TF and mRNA-drug interaction networks were integrated and visualized using Cytoscape.

Fundamental Experimental Techniques

Establishment of Cell Models

Controlled conditions (37 °C, 95% humidity, 5% CO_2) were used to cultivate HNPCs obtained from Wuhan Sunen Biotechnology Co, Ltd in a specialized HNPC complete medium (SNPM-H272; Sunncell, China). Once the cell density reached 80%, the cells were subjected to digestion with a 0.25% trypsin solution and then passed on. In the IVDD model, HNPCs were exposed to 10 ng/mL of IL-1β, while cells that were not stimulated were selected as controls.

Materials and Reagents

Key reagents included: CCK8 Cell Proliferation Assay Kit (BA00208; Bioss, China), HNPC Complete Culture Medium (SNPM-H272; Sunncell, China), Human IL-1 beta Protein (HY-P73149; MedChemExpress, USA), Trypsin (R001100; Gibco, China), RNA Isolation Kit V2 (RC112; Vazyme, China), and antibodies such as Anti-SLPI (ab46763; Abcam, UK) and others from Proteintech and ABclonal.

Experimental RT-qPCR

The cells were placed in 6-well plates at a density of 1×10^6 cells per well and grown at 37 °C in a 5% CO_2 atmosphere. Following incubation, cell lysis was carried out with Buffer RL, and then RNA was extracted and purified. The concentration and purity of RNA were quantified using a NanoDrop 600 instrument. qPCR was performed to evaluate gene expression after cDNA synthesis using a 20-μL reaction setup.

Quantitative Data Analysis

Statistical analyses were conducted using R (version 4.3.1), and the results were reported as the mean ± standard deviation (SD). Comparisons between two groups were conducted using Student *t* test, while multiple group comparisons were performed using one-way analysis of variance (ANOVA). A significance level of $p<0.05$ was utilized. Non-normally distributed data were analyzed using the Mann-Whitney *U* test. Data visualization was performed using GraphPad Prism (version 9.5.1) and ImageJ, with figure preparation undertaken using Adobe Illustrator (2023). A statistical significance level of $p<0.05$, $p<0.01$, and $p<0.001$ was employed.

RESULTS

Recognition and Analysis of Differentially Expressed Genes

To construct a comprehensive view of the expression across diverse datasets, we amalgamated expression profiles from two distinct datasets. Recognizing the pronounced batch effect inherent to datasets from disparate sources, we applied corrections to this batch effect and subsequently normalized the data. Subsequent analyses revealed that the expression distributions of all samples, post-processing, exhibited consistent patterns. This enhanced the precision and reliability of subsequent analyses, as depicted in Figure 1. Using this corrected dataset, we performed differential expression analysis, identifying 112 DEGs. Among these DEGs, 73 were upregulated, and 39 were downregulated (Figure 2).

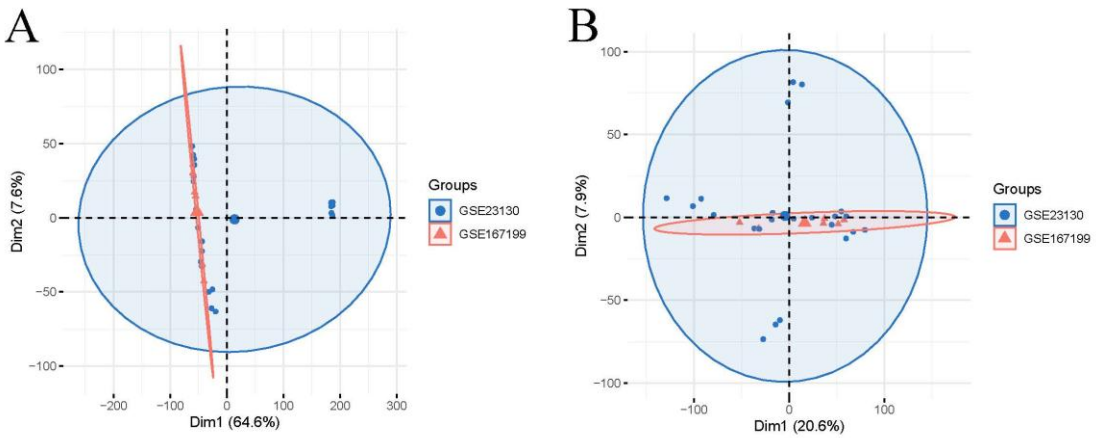

Figure 1. PCA of GSE23130 and GSE167199 before and after batch effect correction and data normalization.

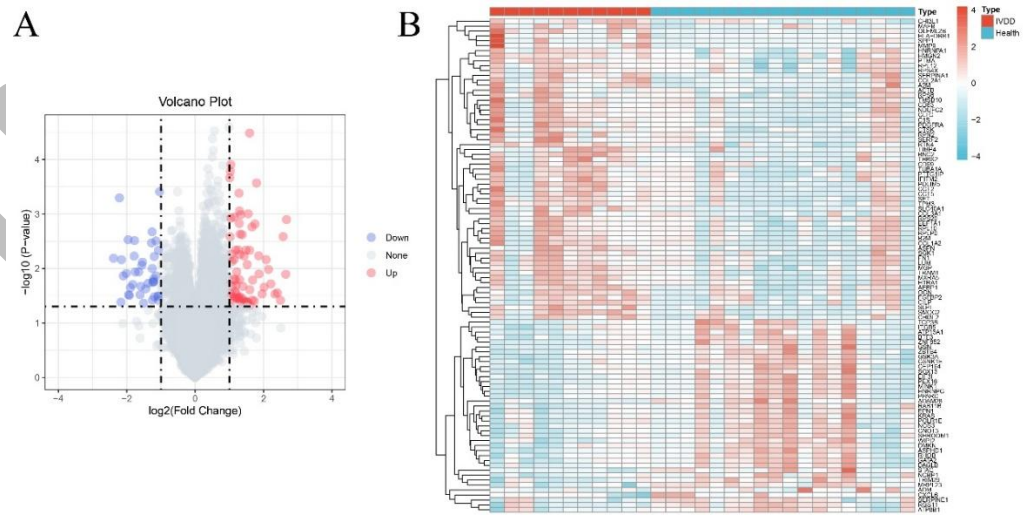

Figure 2. Volcano plot of differentially expressed genes (DEGs). Red represents high expression, blue represents low expression, and gray represents no difference (A). The distinct clustering of Intervertebral Disc Degeneration(IVDD) and control samples in the heatmap (B), driven by these DEGs, underscores a profound alteration in the disc's molecular landscape, which is likely to underpin the observed immunological changes in IVDD.

PPI Network Analysis, GO, and KEGG Enrichment Analysis of DEGs

We undertook a functional enrichment analysis to elucidate the roles of the 112 DEGs in IVDD. Results indicated that the central genes predominantly partake in the biological process (BP) domains such as extracellular matrix organization, cytoplasmic translation, and translation. For the cellular component (CC), they were primarily associated with the extracellular exosome, the extracellular space, and the extracellular region. In terms of molecular function (MF), they were connected to attributes such as the extracellular matrix structural constituent, collagen binding, and protein binding. These central genes were further enriched in KEGG pathways, notably the PI3K-Akt signaling pathway and focal adhesion (Figure 3). Moreover, the PPI network of the 112 DEGs was generated using the STRING database, with Cytoscape software visualizing the DEG interaction network (Figure 4).

Identification of Key Genes by Machine Learning Algorithm

To pinpoint IVDD biomarkers, we employed LASSO regression for feature selection. The regression coefficient path diagram depicted individual gene coefficients. The optimal threshold was chosen at 13 gene coefficients, as evidenced by the cross-validation curve (Figure 5A and 5B). Concurrently, the SVM-RFE algorithm was harnessed to assess central genes (Figure 5C). A Venn diagram illustrated in Figure 5D was used to filter genes common to both algorithms. We discerned 13 pivotal genes for further investigation: *CXCL6*, *CHI3L1*, *RTN4*, *ADM*, *ITGB5*, *TIMP4*, *SLPI*, *BNC2*, *RAB11B*, *CSNK1E*, *MAFB*, *BTF3*, and *SMOC2*.

Immune Infiltration Analysis by ssGSEA

Using normalized data, an immune infiltration analysis was executed to distinguish immune cell infiltration variances between IVDD patients and their healthy counterparts. The ssGSEA method assessed immune infiltration, determining immune cell enrichment levels. Our findings underscored that the infiltration of specific immune cells, notably myeloid-derived suppressor cells (MDSCs) and monocytes, was diminished in the IVDD cohort relative to the control group (Figure 6A and 6B).

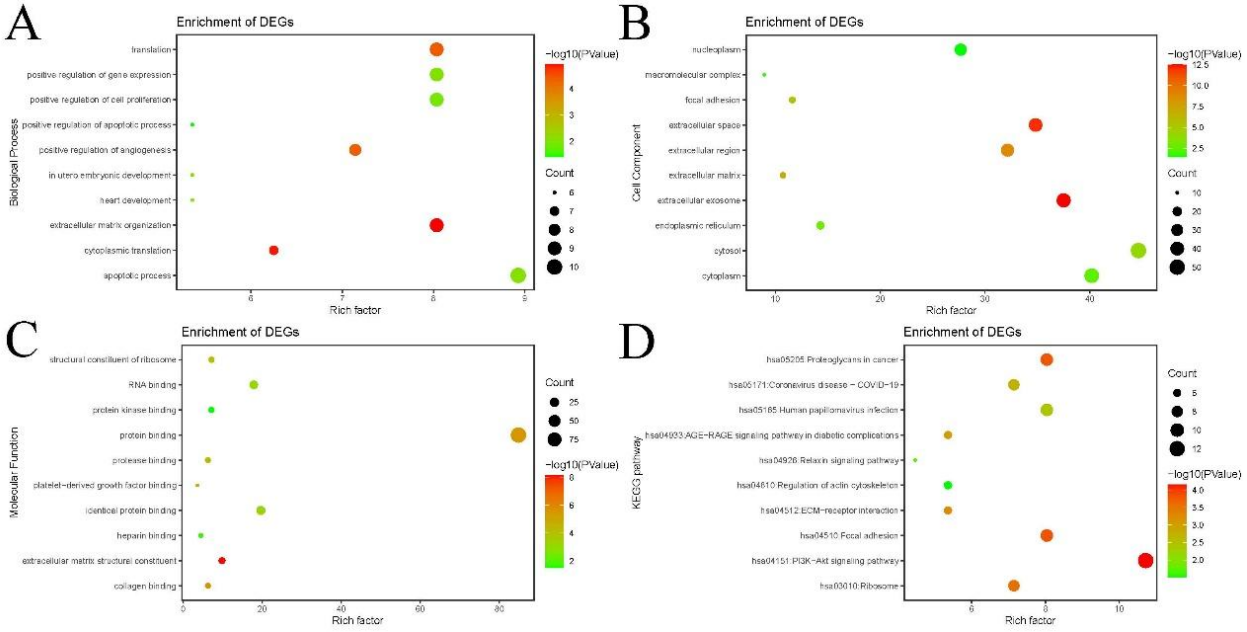

Figure 3. Top 10 terms of GO analysis, including biological process (A), cellular component (B), and molecular function (C). Top 10 terms of KEGG analysis (D). These analyses reveal that DEGs in IVDD are significantly enriched in biological processes and signaling pathways with critical immunological functions.

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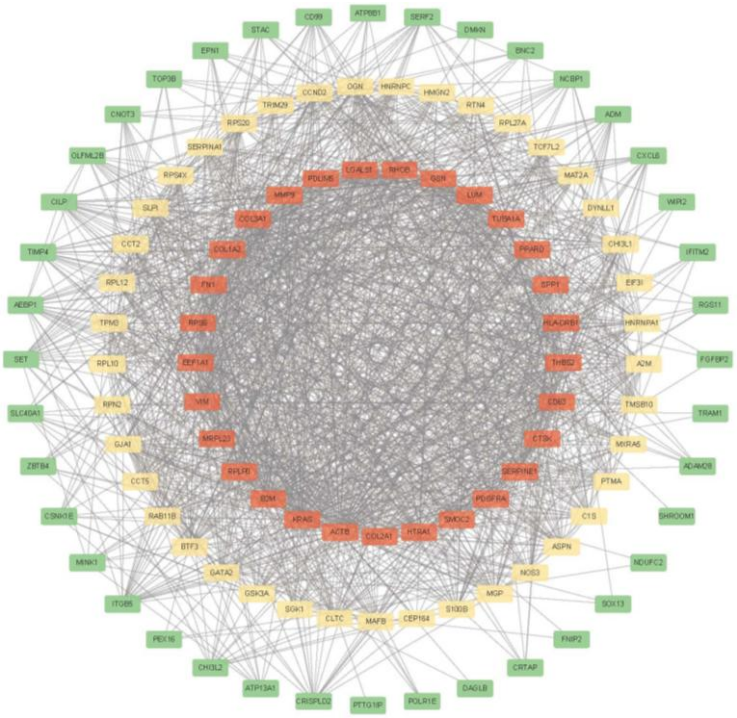

Figure 4. Protein-protein interaction networks of DEGs. Nodes are colored by degree score, transitioning from red (high) to yellow (medium) and green (low).

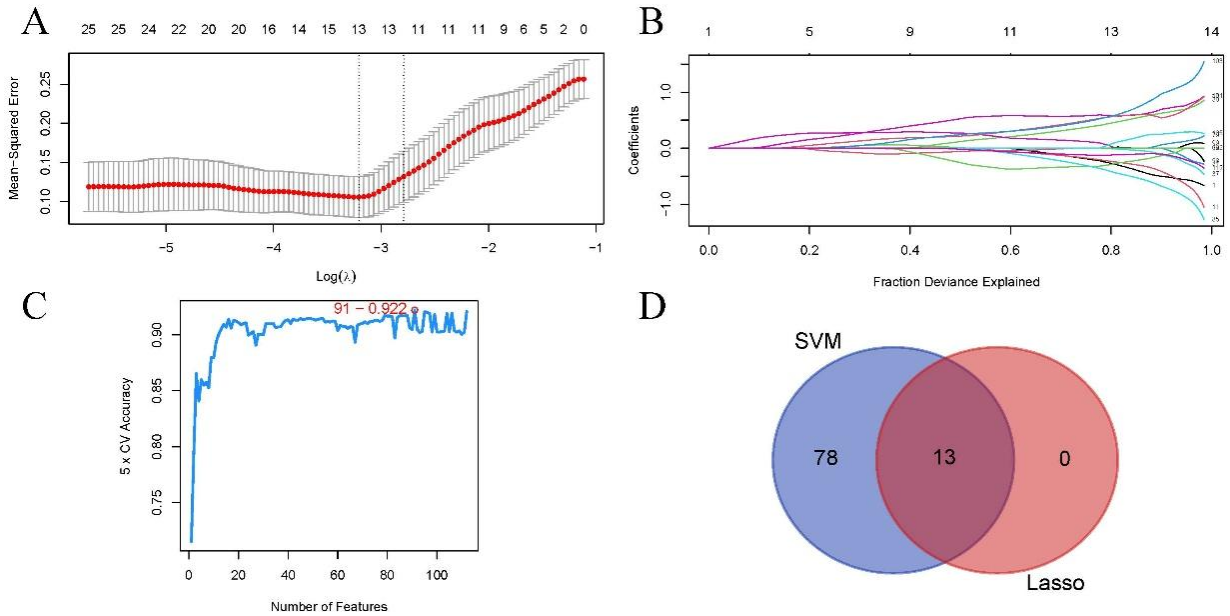

Figure 5. Identification and selection of hub genes. (A) Confidence interval for each lambda; (B) Distribution of lasso coefficients in Lasso regression; (C) SVM feature selection algorithm; (D) the hub genes between Lasso regression and SVM were shown in a Venn diagram. The application of two distinct machine learning algorithms (LASSO and SVM) robustly identifies a set of hub genes. These genes form the basis for subsequent investigations into their specific roles in regulating the immune microenvironment of IVDD.

Immune Cell Infiltration and Key Genes

Delving deeper into potential molecular underpinnings of pivotal genes in IVDD progression, we explored their relationship with immune cell infiltration. Correlation assessments revealed that *RAB11B* and *BTF3* bore significant negative correlations with monocytes, whose infiltration was reduced in IVDD (p<0.05; Figure 6C).

Construction of mRNA-TF Interaction and Identification of Potential Drugs

Our investigation spotlighted possible transcription factor targets for central genes. Using the MotifMap online database, we recognized a total of 351 interactions. The resultant drug–hub gene interaction network was visualized using Cytoscape software (Figure 7). The DGIdb database elucidated connections between central genes and existing or prospective drugs. We identified 18 potential drugs or chemical entities related to 5 mRNAs (Figure 8). Specifically, 7 drugs or chemicals were found to target *ADM*, while 4 were associated with *ITGB5*.

In Vitro Validation of Potential Transcriptional Factor Targets in IVDD

To validate potential transcriptional factor targets involved in IVDD through in vitro experiments, we leveraged the MotifMap online database, identifying a total of 351 interactions. Subsequently, we visualized the drug-hub gene interaction network using Cytoscape software, revealing *ADM*, *ITGB5*, *RTN4*, *SLPI*, and *CSNK1E* as the core targets of these drugs. To establish an in vitro model, HNPCs were utilized. An IL-1β (10 ng/mL) concentration gradient was applied to HNPCs at various time points (0 hours, 12 hours, 24 hours, 36 hours, and 48 hours) using CCK8 assays to optimize the model (Figure 9A). qPCR and Western blot (WB) analyses were then performed to assess the expression levels of *ADM*, *ITGB5*, *RTN4*, *SLPI*, and *CSNK1E*. Compared to the Control group, the expression of *ADM*, *ITGB5*, *RTN4*, *SLPI*, and *CSNK1E* was significantly downregulated in the IVDD group (Figure 9B–C).

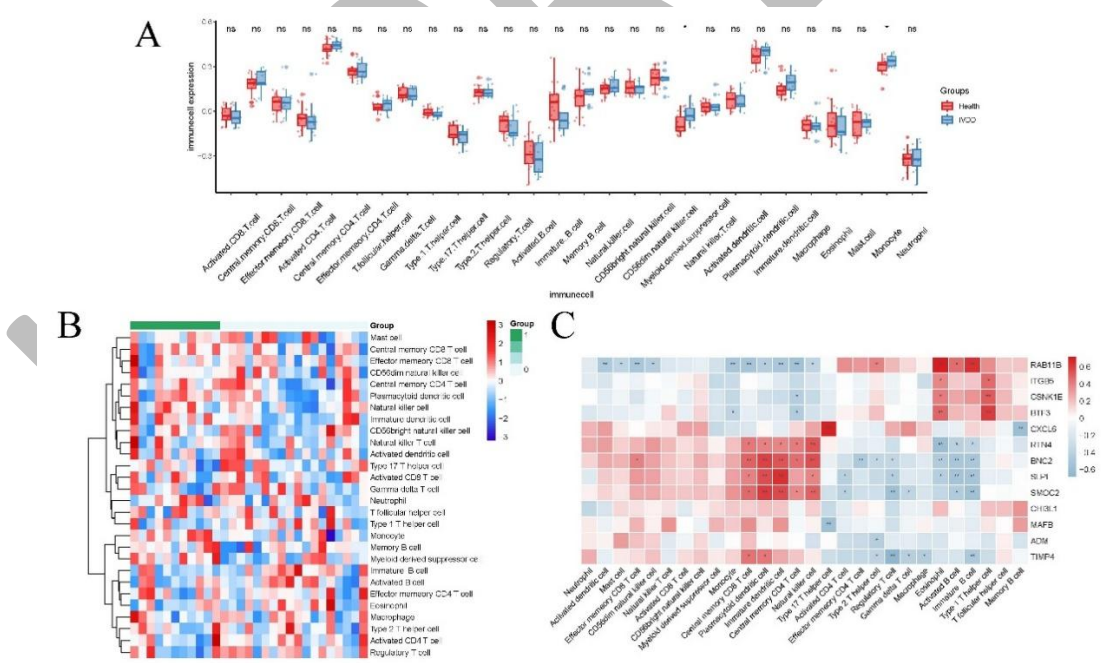

Figure 6. Immune infiltration analysis by ssgsea. A, B. Differences in immune cell expression between the healthy and IVDD groups, the results indicate a significant reduction in the infiltration of Myeloid-Derived Suppressor Cells (MDSCs) and Monocytes in IVDD samples compared to controls, suggesting a potential loss of immunoregulatory and reparative capacity in the degenerative disc microenvironment; C. Correlation between hub genes and 28 different immune cells, *RAB11B* and *BTF3* show significant negative correlations with monocyte abundance, implying a potential link between their downregulation and the reduced monocyte recruitment observed in IVDD.

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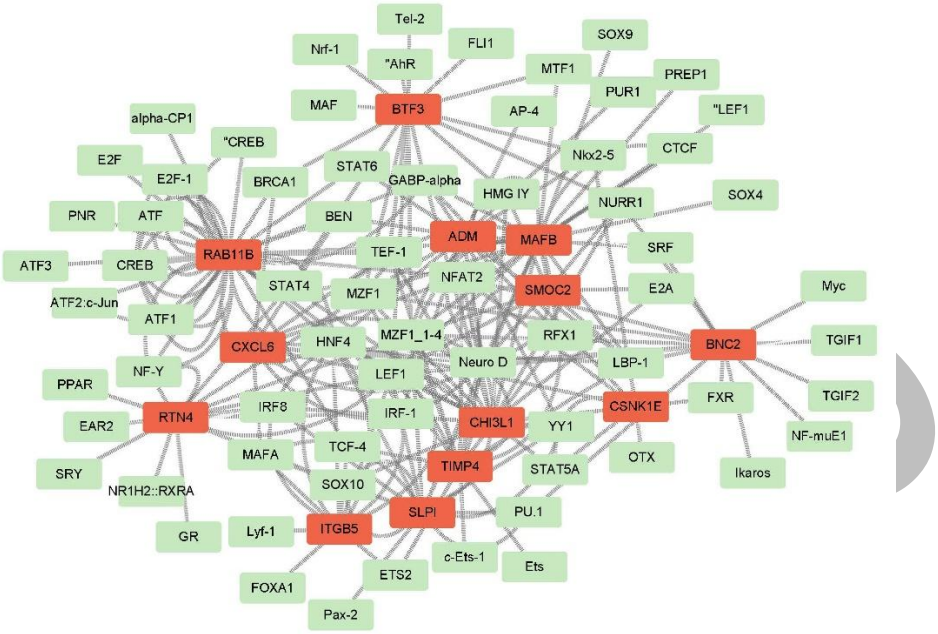

Figure 7. This network predicts the transcription factors that may regulate the expression of hub genes. Identifying these regulators provides insight into the upstream signaling events that could be driving the aberrant gene expression patterns and associated immune dysregulation in IVDD.

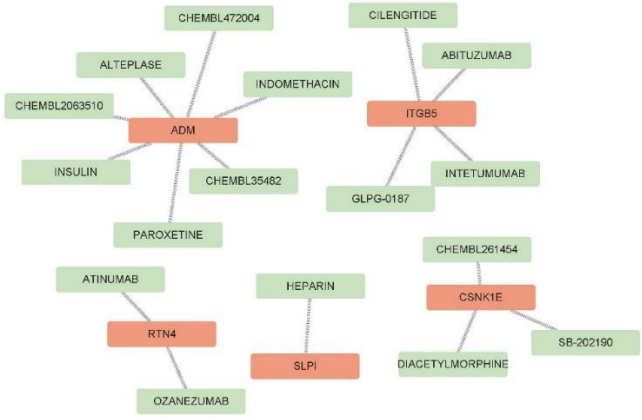

Figure 8. The interaction network between hub genes and potential drugs or chemical molecules, where red nodes represent hub genes, and green nodes represent the drugs or chemical molecules. The interaction map connects hub genes to existing drugs, highlighting potential avenues for drug repurposing in IVDD. This finding underscores the translational potential of targeting these hub genes and their associated pathways to modulate the disc's immune microenvironment for therapeutic benefit.

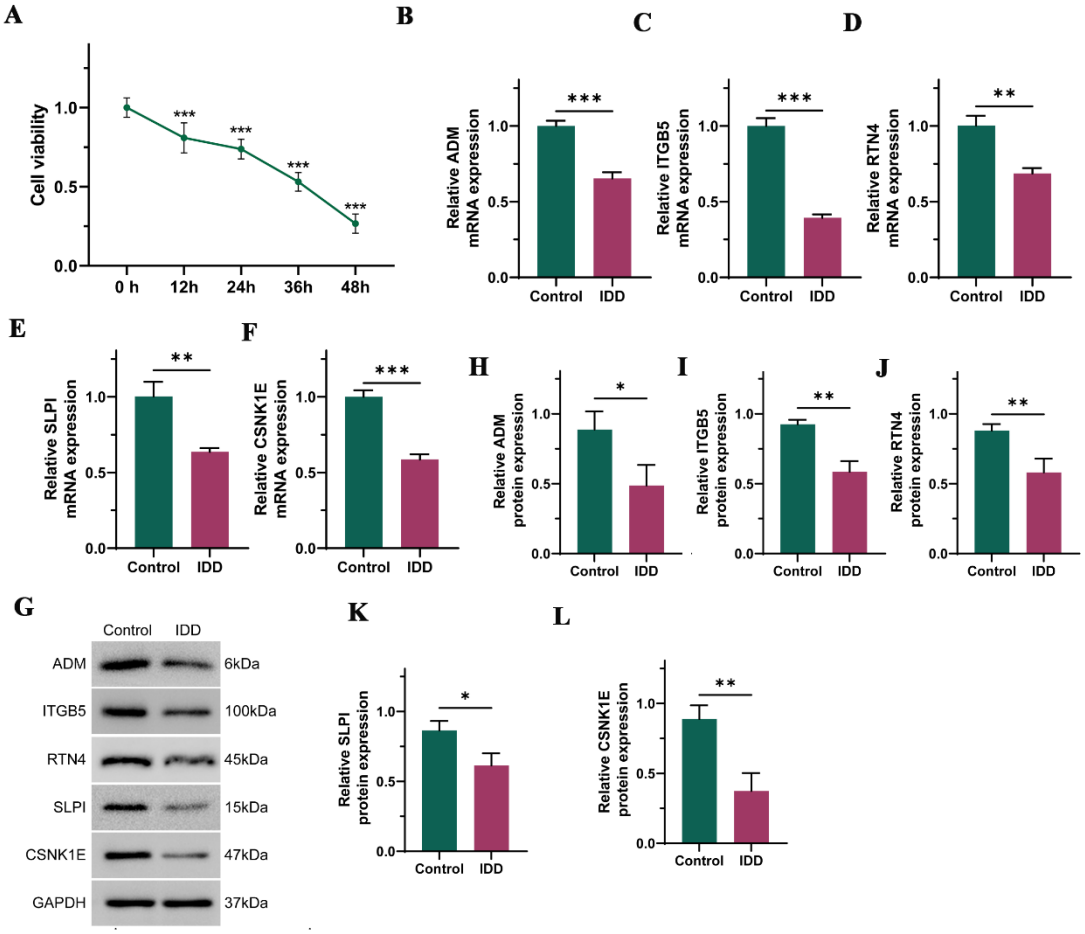

Figure 9. A: Changes in cell viability over different time points after IL-1β treatment, as assessed by CCK8 assay; B-F: Expression levels of *ADM*, *ITGB5*, *RTN4*, *SLPI*, and *CSNK1E* in the Control and IVDD groups detected by q-PCR; G-L: Expression levels of ADM, ITGB5, RTN4, SLPI, and CSNK1E in the Control and IVDD groups detected by WB.

DISCUSSION

The study's comprehensive immune profiling analyzed 28 immune cell types, revealing a more complex and heterogeneous immune microenvironment in IVDD. This approach uncovered the significant involvement of MDSCs and monocytes, cell types that have received comparatively less attention in IVDD pathogenesis. IVDD stands as a major contributor to chronic back pain and spinal disorders, imposing significant socioeconomic burdens. Current treatment methods struggle to halt the progression of IVDD, and diagnostic techniques for IVDD are largely symptom-based and dependent on imaging technologies, making early intervention challenging. Recent research points to promising areas for intervention, such as targeting the Nrf2/Sirt3 pathway,[21] employing advanced imaging techniques like T1ρ and T2* mapping for early-stage detection,[22,] and examining genetic factors that may predispose individuals to IVDD.[23] Investigations into potential therapeutic agents, such as wogonin,[24] extracellular vesicles,[25] and nonviral gene therapy,[26] are also gaining traction. Additionally, growing evidence underscores the involvement of the immune system, particularly macrophages, in IVDD pathology.[12,27–29] While macrophages have been confirmed as key players in IVDD by numerous studies, the role of other immune cell types in IVDD remains largely unexplored.

In this study, we analyzed gene expression datasets GSE23130 and GSE167199 from the GEO database. A

total of 112 DEGs were identified, primarily linked to pathways such as extracellular matrix (ECM) organization, cytoplasmic translation, and the PI3K-Akt signaling pathway. These findings align with earlier reports, such as Gou et al's study, which highlighted significant disruptions in ECM organization and focal adhesion pathways in IVDD patients.[30] Furthermore, the PI3K-Akt pathway has been recognized for its protective role in IVDD, particularly through enhancing ECM integrity and reducing oxidative stress.[31–33] Using LASSO and SVM algorithms, we pinpointed 13 critical genes associated with IVDD, including *CXCL6*, *CHI3L1*, and *ITGB5*. We also examined immune cell infiltration in IVDD, uncovering the involvement of cells like MDSCs and monocytes in IVDD pathogenesis. The analysis extended to transcription factors linked to key genes, highlighting *ADM* and identifying 18 potential drugs that could influence IVDD progression.

Among the identified key genes, *ITGB5*, part of the integrin receptor family, has been linked to various pathological conditions, including cancer and angiogenesis.[34,35] *ITGB5* is involved in processes like virus internalization and the activation of latent transforming growth factor-beta, both of which contribute to fibrosis and related diseases.[36,37] In our study, *ITGB5* was correlated with pathways related to ECM organization, extracellular exosomes, and the PI3K-Akt signaling pathway. This suggests a potential regulatory role for *ITGB5* in IVDD progression. Further research is needed to clarify its role in these pathways and to enhance our understanding of IVDD's underlying mechanisms.

Emerging research has illuminated the connection between immune responses and IVDD.[7,8,27] Nucleus pulposus cells in humans express Fas ligand, which triggers apoptosis in certain immune cells.[38] Additionally, there is increasing evidence that the nucleus pulposus could provoke an autoimmune response.[39] Studies using animal models have shown that exposing nucleus pulposus material to the immune system elicits an immune reaction, and T-cell activation may contribute to autoimmune processes, especially in the context of disc herniation.[40–42] Macrophages, in particular, have been shown to exacerbate disc degeneration and associated pain through the release of inflammatory mediators. In our research, we observed notable infiltration of MDSCs and monocytes in IVDD samples. However, macrophage levels remained stable across both groups. The identified genes, *RAB11B* and *BTF3*, showed strong negative correlations with monocyte levels, consistent with their downregulation. Zhou et al's research aligns with our findings, revealing significant changes in peripheral MDSCs in advanced lumbar disc herniation.[43] Monocytes have also been identified as critical players in the onset, progression, and remission of IVDD.[39] Furthermore, adaptive immunity likely contributes to IVDD, where a T_H17/Treg imbalance could drive persistent inflammation. Thus, directly isolating and quantifying these lymphocyte subsets from disc tissues is a crucial next step. As our understanding of immune cell involvement in IVDD deepens, we anticipate new, targeted therapies that could improve outcomes for patients suffering from back pain due to disc degeneration.

Our analysis using the DGIdb database revealed 18 potential therapeutic agents associated with 5 key mRNAs, including drugs such as alteplase, indomethacin, and insulin. Specifically, 7 of these compounds target *ADM*, while 4 are linked to *ITGB5*. ADM, a multifunctional peptide hormone, plays a role in various biological processes and has anti-inflammatory properties, influencing immune responses.[44–47] Given that inflammation is central to IVDD pathogenesis, *ADM* may influence the progression of the disease through its interaction with immune cells. *ITGB5*, associated with ECM organization and PI3K-Akt signaling, further supports its potential as a therapeutic target. Although existing literature on *ADM* and *ITGB5* in IVDD is limited, our findings suggest these genes could offer promising avenues for treatment. Further studies are essential to verify the therapeutic potential of the identified drugs in IVDD treatment. From a therapeutic perspective, our immune infiltration findings suggest that targeted immunomodulation, rather than broad immunosuppression, could be a viable strategy for IVDD. For instance, if the deficit in MDSCs is validated as a key pathological event, therapeutic approaches aimed at promoting their expansion or recruitment.

The observed reduction in MDSC and monocyte infiltration suggests a deficit in immunoregulatory and reparative capacity, rather than solely an overactive inflammatory response, may drive IVDD progression.[48] The loss of immunosuppressive MDSCs could permit sustained inflammation, accelerating matrix degradation and cellular senescence, while diminished monocyte recruitment might impair tissue repair.[49] The unchanged overall macrophage abundance, despite their established

role, hints that functional polarization (e.g., a pro-inflammatory M1 shift) may be more critical than total numbers—a nuance beyond the resolution of our bulk transcriptomic data. These patterns share conceptual parallels with other musculoskeletal disorders like osteoarthritis, yet the specific reduction in MDSCs may be a unique feature of IVDD.[50,51] Furthermore, this local immune dysregulation must be considered within the context of systemic inflammatory states, such as aging or obesity, which can precondition the disc and amplify local responses.[52,53] Thus, IVDD pathogenesis appears to involve a complex interplay between a deficient local immunosuppressive microenvironment and potentiating systemic inflammation.

In summary, the principal insight of our work is the critical role of immune cell heterogeneity in IVDD. We demonstrate that disease progression is characterized by a loss of specific immunoregulatory subsets, particularly MDSCs and monocytes, creating a permissive environment for sustained inflammation and tissue breakdown. The hub genes we pinpointed offer mechanistic links to this immune dysregulation. This paradigm shift-understanding IVDD as a failure of immune homeostasis-directly points to novel, targeted therapeutic strategies aimed at rebalancing the disc's immune landscape, moving beyond conventional approaches.

While this study provides valuable insights, it is not without limitations. Although we employed cross-validation to reduce overfitting in our machine learning models, the potential for overfitting remains a general consideration in feature selection studies. The external validation of these hub genes in independent cohorts is essential to confirm their generalizability. More in-depth studies are needed to explore the protein-level interactions of these key genes. Additionally, validation of gene expression findings in clinical samples is crucial. Future research should focus on uncovering the detailed molecular mechanisms underpinning IVDD and exploring the interactions between the identified genes and their associated signaling pathways. Regarding the in vitro validation, the experiments were conducted using a single commercial source of HNPCs and lacked replication across multiple primary cell lines from different donors. This limits the generalizability of the validation results and warrants confirmation in a broader set of primary cells. These investigations will ultimately provide a more robust foundation for improving clinical diagnostics and therapeutic options for IVDD.

STATEMENT OF ETHICS

Not applicable.

FUNDING

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGMENTS

Not applicable.

DATA AVAILABILITY

The datasets analyzed during the current study are publicly available in the GEO repository (GSE23130 and GSE167199). The data supporting the findings of this study are available from the corresponding author upon reasonable request.

AI ASSISTANCE DISCLOSURE

Not applicable.

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