Oncology Treatment Discovery, 2025, Volume 3, Issue 4
http://ojs.bbwpublisher.com/index.php/OTD
Online ISSN: 2981-8079
Print ISSN: 3083-4996

From Mendelian Randomization to Bioinformatics Analysis: The Bridging Role of NOD2 in the Relationship Between Crohn's Disease and Pancreatic Cancer

Bingxu Gu*

Department of Hepatobiliary Surgery, Guangzhou Institute of Cancer Research, the Affiliated Cancer Hospital, Guangzhou Medical University, Guangzhou 510000, China

**Corresponding author: Bingxu Gu, 15163602961@163.com*

Abstract: *Background*: Pancreatic cancer (PC) is a leading cause of cancer death worldwide, with early diagnosis being difficult and prognosis poor. Crohn's disease (CD) is a chronic inflammatory bowel disease, and some studies suggest a potential link between CD and the development of pancreatic cancer. However, the exact biological mechanisms are unclear. This study investigates the causal relationship between CD and PC and focuses on the role of the NOD2 gene in pancreatic cancer. *Methods*: The study used Mendelian randomization (MR) to identify SNPs associated with both CD and PC, followed by functional annotation through the Ensembl database. Differential expression of these genes in pancreatic cancer was analyzed using the GEPIA2 platform. The study then used Metascape for gene enrichment and pathway analysis, and Kaplan-Meier Plotter to assess the relationship between gene expression and patient survival. Immunohistochemistry (IHC) was conducted to validate protein expression, and the TIMER 3.0 platform was used to examine immune cell infiltration related to NOD2. Finally, the study explored the relationship between NOD2 mRNA expression and clinical features using the cBioPortal platform. *Results*: Out of 91 candidate genes, 36 showed significant differential expression between pancreatic cancer and normal tissues. High expression of 9 genes was associated with poor prognosis. NOD2 was identified as a key gene with elevated expression in pancreatic cancer tissues, closely linked to immune cell infiltration. Further analysis showed that NOD2 expression correlated with tumor stage, lymph node metastasis, and distant metastasis, especially in advanced stages (T3, N1, Stage IIB). *Conclusion*: This study highlights the potential role of the NOD2 gene in linking Crohn's disease with pancreatic cancer, suggesting that NOD2 may contribute to pancreatic cancer development through immune and inflammatory processes. Elevated NOD2 expression is associated with clinical features of pancreatic cancer, making it a potential prognostic marker. Future research should focus on understanding NOD2's role in the immune microenvironment and its potential as a therapeutic target.

Keywords: Pancreatic cancer; Crohn's disease; Mendelian randomization; NOD2; Immune infiltration

Online publication: December 31, 2025

1. Introduction

Pancreatic Cancer (PC) is one of the leading causes of cancer-related mortality worldwide. It has a low rate of early diagnosis and a high recurrence rate, which leads to a very poor clinical prognosis. According to global cancer statistics, the five-year survival rate for pancreatic cancer is approximately 10%, with most patients diagnosed at advanced stages. Therefore, effective early screening and treatment methods have not yet made significant progress [1]. Current research focuses on revealing the molecular characteristics of pancreatic cancer to identify new diagnostic biomarkers and therapeutic targets, with the aim of improving patient survival.

Crohn's Disease (CD) is a chronic inflammatory bowel disease characterized by persistent, non-specific inflammation in the intestines. The etiology is complex and likely closely related to genetic, immune, and environmental factors [2]. Patients with CD are affected by abnormal immune system responses, which can lead to an imbalance between intestinal tissue damage and repair. Recent studies have suggested that CD may be linked to certain cancers, particularly those in the digestive system [3]. While research has explored the relationship between CD and colorectal cancer [4], the association between CD and pancreatic cancer has not been fully explained. Given the shared characteristics of chronic inflammation and immune system abnormalities between CD and pancreatic cancer, this provides a theoretical basis for further exploration of the potential biological relationship between the two.

A recent study using Mendelian randomization analysis explored the causal relationship between CD and pancreatic cancer [5], revealing a possible causal connection. The study indicated that CD is a risk factor for pancreatic cancer. However, despite providing preliminary evidence for the relationship between CD and pancreatic cancer, the biological mechanisms involved remain unclear.

This study aims to further explore the causal relationship between CD and pancreatic cancer and uncover potential molecular mechanisms. By integrating existing genetic data, gene functional annotations, differential gene expression analysis, and enrichment analysis, the study hope to identify candidate genes with potential prognostic value in pancreatic cancer, providing new ideas for future biological research and targeted therapies.

2. Materials and methods

2.1. Overall study design

This study's experimental design aims to further investigate the causal relationship between CD and PC using Mendelian randomization analysis, explore the biological links between these two diseases, and identify potential new therapeutic targets for PC. Initially, relevant SNPs associated with CD and PC are identified from the Mendelian randomization literature. These SNPs are then functionally annotated using the Ensembl database to pinpoint the related genes. The next step involves utilizing the GEPIA2 platform to analyze the significantly differentially expressed genes from the identified genes in PC as candidate genes. Subsequently, the differentially expressed genes undergo enrichment analysis on the Metascape platform to determine the biological processes and signaling pathways in which these genes participate. Following this, survival analysis is conducted using the Kaplan-Meier Plotter tool to identify genes that correlate with overall survival in PC patients. Genes with a P-value < 0.05 and a hazard ratio (HR) > 1 are selected as candidate prognostic markers for PC. A literature review is then conducted to identify potentially unexplored prognostic candidate genes. Immunohistochemical analysis is performed using the Human Protein Atlas database to validate the differential expression of these prognostic candidate genes in both normal pancreatic tissue and PC tissue. Finally, for the selected target genes, immune infiltration analysis is carried out using the TIMER 3.0 platform to evaluate their roles in the immune

microenvironment of PC. Furthermore, the relationship between the mRNA expression of these genes and the clinical pathological features of PC patients is analyzed through the cBioPortal platform (**Figure 1**).

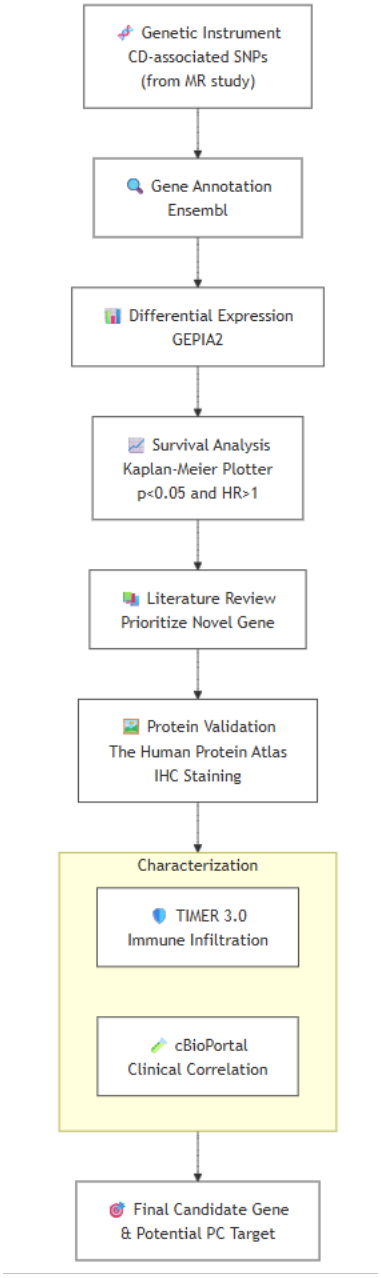

Figure 1. Experimental design flow chart.

2.2. Data sources

The causal relationship analysis between CD and PC discussed in this paper is based on the results of Mendelian randomization studies [5].

2.3. SNP functional annotation analysis

The Ensembl Genome Browser (https://www.ensembl.org/) is a free and publicly accessible genome browsing tool, developed and maintained collaboratively by the European Bioinformatics Institute (EBI) and the Wellcome

Sanger Institute [6]. This platform provides a powerful resource for scientists and researchers worldwide to browse and analyze genomic data across multiple species, with particularly important applications in gene annotation, variant analysis, and transcriptomics. To further explore the relevant SNPs, the study used the Ensembl Genome Browser for functional annotation of the SNPs and to pinpoint related candidate genes.

2.4. Differential expression analysis

GEPIA2 (http://gepia2.cancer-pku.cn/) is an online platform for cancer gene expression analysis and biomarker research [7]. The study performed differential expression analysis on the transcription levels between pancreatic cancer tissue (from the TCGA database) and normal pancreatic tissue (from the GTEx database) using the GEPIA2 platform [10]. The analysis utilized log2(TPM+1) normalized expression values and applied univariate differential testing for statistical analysis.

2.5. Gene enrichment analysis

Metascape is a free online bioinformatics platform that provides researchers with gene functional annotation, enrichment analysis, pathway analysis, and visualization tools, helping users understand the interactions between genes, genomes, and proteins [8]. For the selected differentially expressed genes, the study used the Metascape platform to perform gene enrichment analysis, aiming to identify the enrichment of these genes in biological processes, molecular functions, and cellular components, and to obtain relevant information about associated signaling pathways and biological functions.

2.6. Survival analysis

Kaplan-Meier Plotter is a widely used online tool for analyzing the relationship between gene expression and patient survival, particularly in cancer research [9]. This tool is based on the Kaplan-Meier survival analysis method, which assesses the association between gene expression and overall survival (OS), disease-free survival (DFS), and other clinical outcomes by plotting survival curves. The study used the Kaplan-Meier Plotter tool to conduct clinical prognosis analysis of these differentially expressed genes and evaluate their correlation with overall survival (OS) in pancreatic cancer patients.

2.7. Literature review

The study conducted a literature review on the candidate genes and, in conjunction with existing pancreatic cancer research, selected genes that have not been thoroughly studied as further candidates. These genes have not been fully explored in pancreatic cancer research and are thus considered potential avenues for future study.

2.8. Protein expression validation and immunohistochemical analysis

The Human Protein Atlas is an open, biomedical research platform aimed at providing expression data for all proteins in the human genome, as well as their distribution across different tissues, cells, and pathological states [10]. The platform, initiated and maintained by the Karolinska Institute in Sweden, consolidates extensive experimental data, playing a significant role, particularly in proteomics and clinical pathology research. To validate the expression of the candidate genes in pancreatic cancer, the study used the Human Protein Atlas database to search for and compare the immunohistochemical (IHC) staining results of these genes in normal pancreatic tissue and pancreatic cancer tissue, ensuring that their protein expression levels align with the differential expression results

obtained from the GEPIA2 platform.

2.9. Immune infiltration analysis

TIMER 3.0 (Tumor Immune Estimation Resource) is a powerful online platform for cancer immune microenvironment analysis [11]. It aims to assist researchers in assessing immune cell infiltration in tumors by integrating various cancer datasets and to explore the relationship between the immune microenvironment and cancer initiation and progression. The study performed immune infiltration analysis of the target genes on the TIMER 3.0 platform to evaluate their role in the immune microenvironment of pancreatic cancer.

2.10. Clinical pathological analysis

Finally, the study performed a correlation analysis between the mRNA expression of the target genes and the clinical pathological features (such as staging, prognosis, etc.) of pancreatic cancer patients using the cBioPortal platform, to evaluate the potential role of these genes in the initiation and progression of pancreatic cancer. cBioPortal is a widely used online platform for the visualization, analysis, and interpretation of cancer genomics data [12]. It was developed by the Memorial Sloan Kettering Cancer Center, a public genomic data platform in the United States, and is designed to provide an integrated, interactive tool to assist researchers and clinicians in analyzing genomic, transcriptomic, and clinical data across different cancer types, and their relationship with patient prognosis and treatment response.

3. Results

3.1. SNP functional annotation analysis

Based on the results of Mendelian randomization analysis in the existing literature, 82 SNPs associated with CD and PC were extracted. Functional annotation through the Ensembl database revealed that these SNPs are mapped to 91 genes. This analysis provides foundational data to support further investigation into the role of these genes in PC.

3.2. Differential gene expression

Differential expression analysis was performed on the 91 genes identified from the SNP functional annotation analysis using GEPIA2. The results showed that after filtering, 36 genes exhibited significant expression differences between pancreatic cancer and normal pancreatic tissue. These genes are: ACO2, ADCY3, APEH, ATG16L1, ATXN2, CARD9, CREM, ERAP2, FCGR2A, FOS, FUT2, HLA-B, HLA-DRA, IFNGR2, IKZF3, IL18R1, IRF1, JAK2, JAZF1, LSM14A, MTX1, NFATC1, NOD2, PHF5A, PHTF1, PLA2G4A, PLAU, PRDM1, RASGRP1, RMI2, RNF123, SBNO2, SH2B3, SKAP2, SLAIN2, TAGAP.

3.3. Functional enrichment analysis

3.3.1. Pathway and biological process enrichment analysis

In this enrichment analysis, the top 15 significantly enriched pathways and biological processes were identified. These pathways mainly reflected those related to immune response, inflammation, and viral response (**Figure 2**). The enriched terms were primarily concentrated in immune response, cell activation, and certain diseases such as inflammatory bowel disease, tuberculosis, and leishmaniasis, suggesting that CD may be potentially associated with the development of PC through these pathways. The specific analysis results are as follows:

(1) T Cell Activation and SARS-CoV-2 (WP5098): This pathway included six differentially expressed genes (16.67%) and showed significant enrichment among all input genes. T cell activation is a central process of the adaptive immune response, and chronic immune activation in CD may increase the risk of PC through this mechanism.

(2) Regulation of Adaptive Immune Response (GO:0002819): This process involved seven differentially expressed genes (19.44%) and exhibited a high degree of enrichment. Patients with CD often display abnormal immune activation, and dysregulation of adaptive immune response may represent an important mechanism underlying PC development.

(3) Inflammatory Bowel Disease (hsa05321, KEGG Pathway): This pathway was enriched with five differentially expressed genes (13.89%). As CD itself is a typical inflammatory bowel disease, the enrichment suggests that intestinal inflammatory responses may indirectly influence PC development through systemic immune pathways.

(4) Leishmaniasis (hsa05140, KEGG Pathway): This pathway involved five differentially expressed genes (13.89%). Although leishmaniasis is rarely directly related to PC, its immune evasion mechanisms may provide biological insight into immune escape in PC.

(5) Regulation of Cell Activation (GO:0050865): This process was enriched with nine differentially expressed genes (25%), indicating that immune cell activation and its regulation may play critical roles in the pathogenesis of PC.

(6) Tuberculosis (hsa05152, KEGG Pathway): This pathway was enriched with six differentially expressed genes (16.67%). As tuberculosis is a chronic immune-mediated disease, this suggests that immune system involvement in chronic inflammatory disorders may influence PC progression.

(7) Response to Virus (GO:0009615): This process involved six differentially expressed genes (16.67%). The relationship between viral infection and cancer has been widely reported, and the persistent immune activation in CD may lead to abnormal antiviral responses, thereby promoting the development of PC.

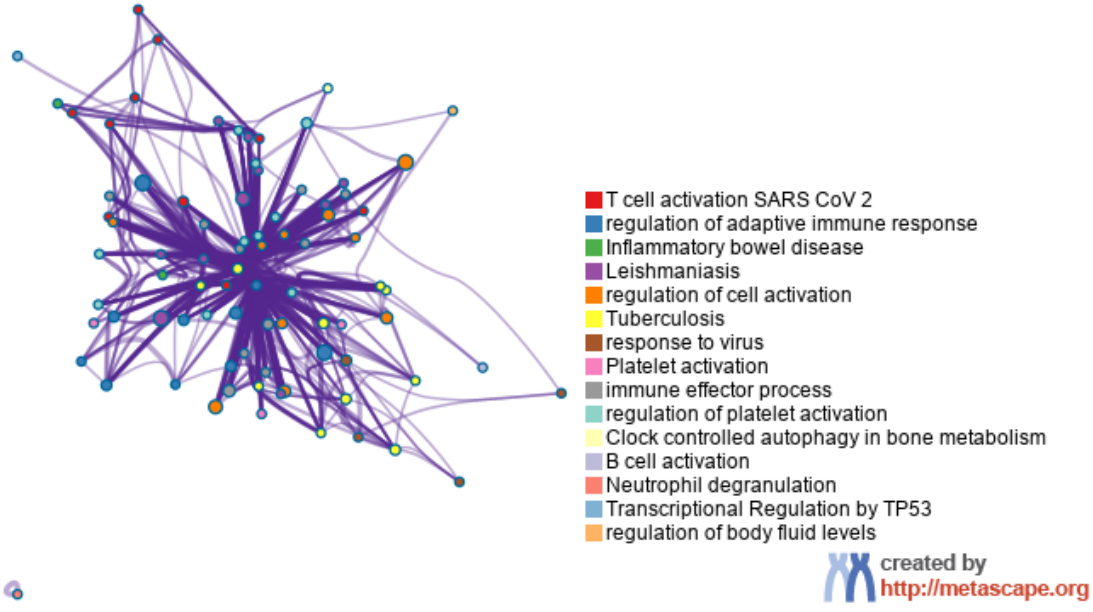

Figure 2. Top 15 enriched pathways and biological processes.

3.3.2. Protein-Protein Interaction (PPI) enrichment analysis

In the PPI enrichment analysis, the significant pathways identified were primarily related to T cell differentiation, regulation of cell activation, and the interferon-γ signaling pathway. The specific analysis results are as follows:

(1) Th1 and Th2 Cell Differentiation (hsa04658): The Log10(P) value for this pathway was -8.6, showing significant enrichment of the differentially expressed genes. Th1 and Th2 cells are two major T cell subpopulations involved in immune responses. CD may promote PC development by altering the balance of these T cell subpopulations.

(2) Regulation of Cell Activation (GO:0050865): The Log10(P) value for this pathway was -8.5, indicating significant enrichment of the differentially expressed genes in cell activation regulation. Activation of immune cells is fundamental to immune responses, and excessive or dysregulated activation of immune cells may influence the immune microenvironment of PC.

(3) Interferon-γ Signaling Pathway (R-HSA-877300): The Log10(P) value for this pathway was -8.5. Interferon-γ is an important immune cytokine, and aberrant activation of the interferon-γ signaling pathway may lead to immune tolerance or immune evasion, thus influencing PC development.

3.3.3. Quality control and association analysis

In the quality control and association analysis section, the results revealed multiple enriched pathways related to immune cell types and tissues, suggesting that these immune cells may play an important role in the relationship between CD and PC. The specific analysis results are as follows:

(1) MANNO MIDBRAIN NEUROTYPES HMGL (M39051): Enrichment analysis showed 10 differentially expressed genes (28%) in this pathway, with a Log10(P) value of -9.00, suggesting that midbrain immune system-associated cells may be involved in the immune microenvironment of PC.

(2) HU FETAL RETINA MICROGLIA (M39266): This pathway was enriched with seven differentially expressed genes (19%), with a Log10(P) value of -6.50, indicating that microglial cells may be involved in regulating the immune microenvironment of PC.

(3) TRAVAGLINI LUNG EREG DENDRITIC CELL (M41697): This pathway was enriched with eight differentially expressed genes (22%), with a Log10(P) value of -6.50. Dendritic cells play an important role in immune responses and may be involved in the immune dysregulation induced by CD, thereby increasing the risk of PC.

3.3.4. Enrichment analysis of the DisGeNET database

In the enrichment analysis of the DisGeNET database, the significant disease pathways were primarily concentrated in the areas of immune system abnormalities and autoimmune diseases, revealing potential associations between CD and other immune-mediated diseases with PC (**Figure 3**). The analysis results suggest that the long-term activation of the immune system and chronic inflammatory responses may be key mechanisms by which CD increases the risk of PC. Specifically, the enrichment of immune-mediated chronic disease pathways, such as sclerosing cholangitis, ankylosing spondylitis, and celiac disease, indicates that immune dysregulation is closely related to the development of PC. In particular, the immune mechanisms of CD may promote immune evasion mechanisms in PC through chronic inflammation and immune responses. Furthermore, the enrichment of immune deficiency and immune cell-related pathways, such as common variant immune deficiencies and eosinophil counts, further supports the role of the immune system in immune evasion in PC.

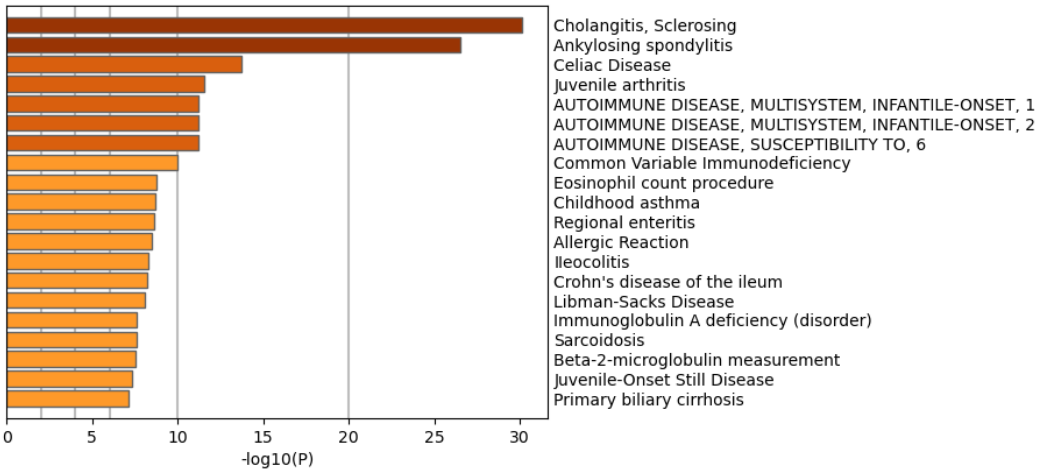

Figure 3. Disease pathways enriched in the DisGeNET database.

3.4. Survival analysis of candidate genes, literature review, and protein validation, screening of target genes

In the preliminary study, differential expression analysis of these genes in pancreatic cancer tissue was performed using the GEPIA2 platform, resulting in the identification of 36 differentially expressed genes. To further validate the prognostic value of these genes in PC, survival analysis was conducted.

3.4.1. Survival analysis

The study used the Kaplan-Meier Plotter platform to assess the overall survival (OS) of the 36 differentially expressed genes. The results showed that high expression of 9 genes was significantly associated with poor prognosis in pancreatic cancer patients ($p < 0.05$, HR > 1). These genes are: ACO2, ATG16L1, ERAP2, NOD2, PHF5A, PLAU, RMI2, SKAP2, and SLAIN2.

3.4.2. Literature review and protein expression validation

To further confirm the biological significance of these candidate genes in PC, the study conducted a literature review. ERAP2, PHF5A, PLAU, and SLAIN2 have already been extensively studied in PC, so they were excluded from further analysis. Subsequently, the study used The Human Protein Atlas database to validate the protein expression levels of the remaining candidate genes in normal pancreatic tissue and pancreatic cancer tissue. The results showed that the protein expression levels of ACO2, ATG16L1, RMI2, and SKAP2 did not show significant differences between normal pancreatic tissue and pancreatic cancer tissue, whereas NOD2 expression was significantly higher in pancreatic cancer tissue compared to normal pancreatic tissue. Based on the results from differential expression analysis, survival analysis, and protein expression validation (**Figures 4 and 5**), the study ultimately identified NOD2 as the target gene for further research.

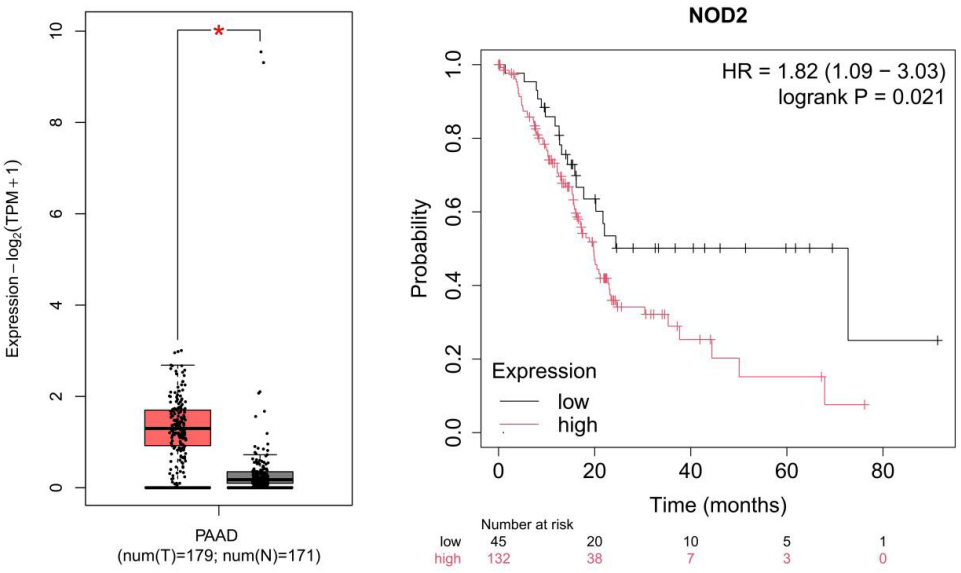

Figure 4. Differential expression and survival analysis results of NOD2.

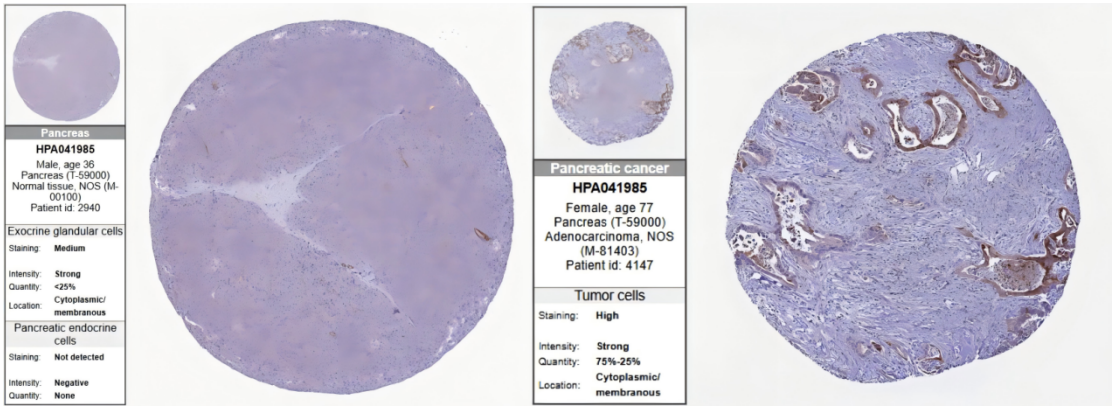

Figure 5. Protein expression level differences of NOD2 in normal pancreatic tissue and pancreatic cancer tissue.

3.5. SH2B3 immune infiltration analysis

The study analyzed the immune infiltration of the NOD2 gene in pancreatic cancer. The results showed that the expression of NOD2 in the pancreatic cancer immune microenvironment is closely related to the infiltration of various immune cell types (**Figure 6**). Specifically, the expression of NOD2 was significantly positively correlated with the infiltration of CD8+ T cells (Rho = 0.377, *P* = 3.95e-07), M2 macrophages (Rho = 0.423, *P* = 8.91e-09), dendritic cells (Rho = 0.604, *P* = 2.67e-18), cancer-associated fibroblasts (Rho = 0.453, P = 5.32e-10), and neutrophils (Rho = 0.548, *P* = 1.00e-14). These results suggest that the NOD2 gene may play a key role in immune surveillance and antitumor immune responses; it may regulate immune evasion mechanisms in the tumor microenvironment by promoting the infiltration of immunosuppressive immune cells (such as M2 macrophages and cancer-associated fibroblasts), thereby influencing the progression of PC.

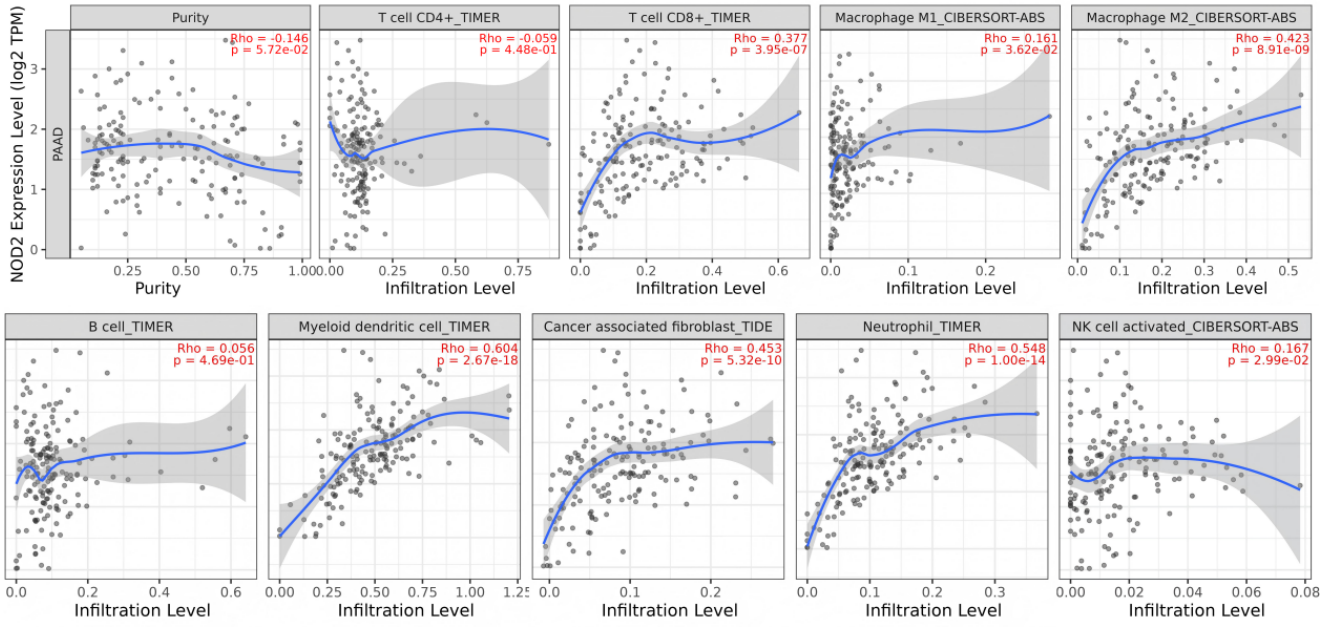

Figure 6. Immune infiltration analysis of NOD2 in pancreatic cancer.

3.6. NOD2 Clinical pathological correlation analysis

In this study, the expression pattern of the NOD2 gene in different clinical pathological stages of pancreatic cancer to determine its correlation with the clinical pathological features of PC (**Figure 7**).

3.6.1. AJCC pathological T stage (Tumor invasion depth)

According to the AJCC pathological T stage, pancreatic cancer is divided into four stages: T1, T2, T3, and T4. The mRNA expression of the NOD2 gene showed significant differences across the different T stages. Specifically, the mRNA expression level of NOD2 was highest in T3 stage pancreatic cancer samples, while the expression levels were relatively low in T1 and T2 stages, and significantly decreased in T4 stage. Regarding gene variation, amplifications (Amplification, red dots) and copy number gains (Gain, pink dots) of NOD2 were observed in all T stages. Particularly in T3, the distribution of NOD2 gene variations was denser, suggesting that the genetic changes in tumor cells at this stage are more complex.

3.6.2. AJCC pathological N stage (Lymph node metastasis)

In the AJCC pathological N stage, pancreatic cancer is divided into N0 (no metastasis), N1 (regional metastasis), N1b, and NX (stage unknown). The expression of NOD2 showed significant differences across the different N stages. Specifically, the expression of NOD2 was highest in N1 stage, followed by the N0 stage, while expression in the NX stage was significantly lower. Additionally, gene variations were more frequent in N0 and N1 stages, where gene amplifications and copy number gains were observed, while no gene variations were observed in the NX stage, suggesting fewer genetic changes in pancreatic cancer at this stage.

3.6.3. AJCC pathological M stage (Distant metastasis)

The M stage is used to assess whether pancreatic cancer has distant metastasis and is divided into M0 (no metastasis), M1 (metastasis present), and MX (stage unknown). The expression of NOD2 showed some variation

across the different M stages. In both M0 and M1 stages, the mRNA expression levels of NOD2 were higher, suggesting that the NOD2 gene may be closely related to the metastasis process in these stages. However, in the MX stage, NOD2 expression was significantly lower, indicating that there may be unknown biological mechanisms at play in pancreatic cancer at this stage. Gene variation analysis showed that in M0 and M1 stages, NOD2 amplifications, copy number gains, and some missense mutations (Missense, VUS, green dots) were more common, while gene variations were almost absent in the MX stage.

3.6.4. AJCC overall pathological stage

For the AJCC overall pathological stage (Stage I, Stage IA, Stage IB, Stage IIA, Stage IIB, Stage III, Stage IV), the study analyzed the expression of NOD2 across the different stages. The results showed that in Stage IIB, NOD2 had the highest mRNA expression level, showing strong gene activity. In Stage I, IA, IB, and IIA, the expression of NOD2 was slightly lower but still significantly higher than in Stage III and Stage IV. Further analysis of gene variation revealed that NOD2 gene variations (including amplifications, gains, and missense mutations) were most densely distributed in Stage IIB pancreatic cancer samples, suggesting that tumors at this stage may have a more complex genetic background.

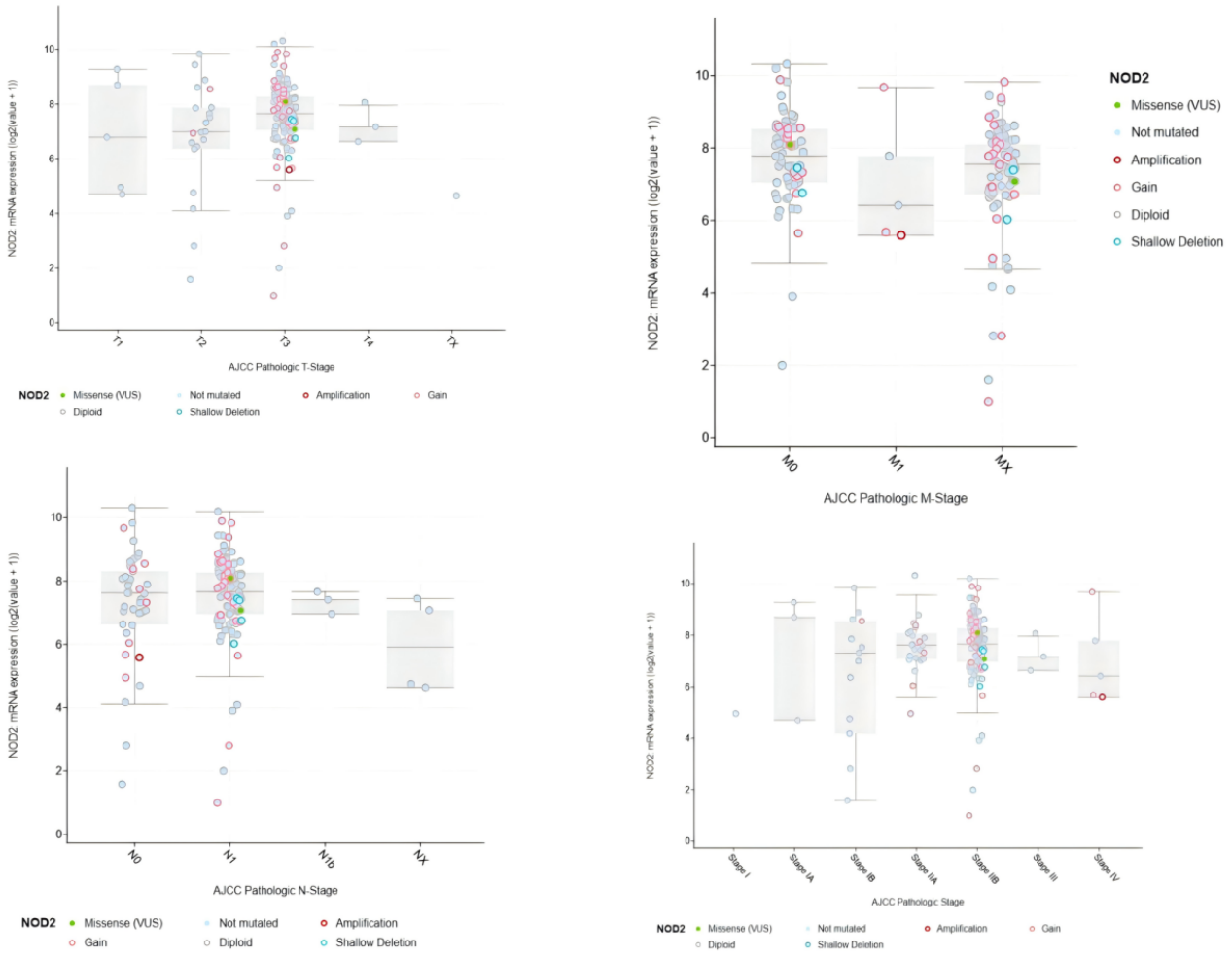

Figure 7. Correlation analysis of NOD2 mRNA expression in different pathological stages of pancreatic cancer.

4. Discussion

This study took the results of Mendelian randomization analysis between CD and PC as a starting point, combining multi-dimensional bioinformatics tools. By analyzing 82 SNPs associated with CD and PC, and performing functional annotation through the Ensembl database, the study identified 91 potential candidate genes. Subsequently, the differential expression of these genes in pancreatic cancer tissue was analyzed using the GEPIA2 platform, ultimately identifying 36 genes that showed significant expression differences between pancreatic cancer and normal pancreatic tissue.

Further enrichment analysis of the differentially expressed genes revealed several pathways significantly associated with immune response, cell activation, inflammation, and pathological responses, suggesting that CD may be closely related to PC through immune pathways. As CD is a chronic immune-mediated inflammatory disease, the sustained abnormal activation of its immune system may contribute to the imbalance of the immune environment, thereby providing favorable conditions for PC development. In particular, the abnormal activation of T cells and the loss of immune tolerance may promote tumorigenesis. Additionally, the enrichment of pathways related to inflammatory bowel disease further supports the potential role of chronic inflammation in CD patients, indicating that the systemic immune response associated with CD may influence the mechanisms underlying PC development. The enrichment of viral response pathways may suggest abnormalities in immune responses in CD patients, potentially leading to immune evasion of viral infections and thus creating an immune escape microenvironment conducive to the development of PC. These results offer new insights into the potential biological mechanisms linking CD and PC, particularly regarding the effects of immune responses and chronic inflammation on PC.

PPI Enrichment Analysis further revealed several important pathways closely related to immune response, providing further support for the hypothesis that CD affects PC development through immune pathways. Specifically, the enrichment results of Th1 and Th2 cell differentiation suggest that the immune system in CD may cause immune dysregulation by altering the balance of T cell subpopulations. This imbalance in immune cell function could provide a favorable immune environment for the development of PC, promoting tumor cell survival and expansion. Furthermore, the enrichment of the cell activation regulation process further revealed that excessive activation of immune cells could lead to changes in the immune microenvironment, thereby facilitating immune evasion in tumors. The enrichment of the interferon-γ signaling pathway suggests that aberrant activation of the interferon-γ signaling pathway may be closely related to immune tolerance and immune evasion mechanisms.

The results from quality control and association analysis also highlighted the crucial role of immune cells. The diversity of immune cells and immune regulation imbalance, particularly the enrichment of dendritic cells, NK cells, and macrophages, reflect the complex role of the immune system in the relationship between CD and PC. The functional imbalance of these immune cells may be an important mechanism by which CD leads to PC.

The enrichment analysis of the DisGeNET database suggests that the abnormal activation of the immune system, chronic inflammatory responses, and autoimmune diseases may be key mechanisms by which CD triggers PC. The long-term activation of the immune system and chronic inflammation may promote the development of PC. These findings provide strong support for further exploration of the role of the immune system in PC and offer insights for clinical immunotherapy research.

Among the numerous differentially expressed genes, NOD2 attracted particular attention. NOD2 is a key pattern recognition receptor predominantly expressed in intestinal epithelial cells and immune cells. It activates the downstream NF-κB pathway through the recognition of muramyl dipeptide (MDP) in bacterial cell walls, thus

regulating immune and inflammatory responses [13]. The dysfunction of NOD2 is closely related to the onset of CD [14]. Common mutations in NOD2 in CD patients (such as R702W, G908R, and 3020insC) lead to functional deficiencies in immune responses, resulting in chronic intestinal inflammation, which is considered a potential trigger for colorectal cancer [15].

Although the role of NOD2 in CD has been extensively studied, there is insufficient evidence regarding its function in PC. Existing studies have shown that NOD2 plays an important role in other types of cancer (such as colorectal cancer, breast cancer, and lung cancer) [16, 17, 18], mainly by affecting immune system functions and changes in the microenvironment, thereby promoting cancer development and progression. In colorectal cancer, NOD2 affects the progression of intestinal carcinogenesis by regulating intestinal immune responses and microbial communities. This mechanism may also play a role in PC development, particularly through influencing the immune microenvironment of the pancreas.

Through the analysis of NOD2 gene immune infiltration in pancreatic cancer, the study found that the expression of NOD2 in the pancreatic cancer immune microenvironment was significantly associated with the infiltration of various immune cell types. Specifically, NOD2 expression showed a significant positive correlation with the infiltration of CD8+ T cells, M2 macrophages, dendritic cells, cancer-associated fibroblasts, and neutrophils. These results suggest that NOD2 may regulate immune evasion mechanisms in the tumor microenvironment by promoting the infiltration of immunosuppressive immune cells (such as M2 macrophages and cancer-associated fibroblasts), thus influencing the progression of PC. Particularly, M2 macrophages and cancer-associated fibroblasts, which commonly play immunosuppressive roles in the tumor immune microenvironment, promote tumor growth and metastasis. Therefore, NOD2 may enhance immune evasion and support tumor growth by regulating the function of these immune cells.

In the pathological correlation analysis, the study explored the expression pattern of NOD2 in different clinical pathological stages of pancreatic cancer and its relationship with clinical features of PC. The results showed significant differences in the expression of NOD2 across different pathological stages, particularly in the T stage, N stage, and M stage. In the T stage, NOD2 expression was highest in T3, while it was significantly reduced in T4, suggesting that NOD2 may play a key role in stages with greater tumor invasion depth. Additionally, gene variations were more densely distributed in T3, reflecting the more complex genetic changes in tumor cells at this stage. Regarding the N stage, NOD2 expression was highest in N1, and gene variations (such as amplifications and copy number gains) were more common in N0 and N1, indicating that NOD2 may be closely related to lymph node metastasis in PC. In the M stage, NOD2 expression was higher in both M0 and M1, suggesting a strong association with distant metastasis. In the AJCC overall pathological stage, NOD2 showed the highest expression in Stage IIB, with a denser distribution of gene variations, indicating a more complex genetic background in tumors at this stage.

Although this study reveals the potential role of NOD2 in the relationship between CD and PC, there are still some limitations. Firstly, these results need further validation through laboratory research and clinical samples. Secondly, this study primarily relies on publicly available databases and bioinformatics analysis, lacking direct clinical data support. Therefore, future research should focus on experimental validation of NOD2's role in PC through clinical samples and animal models, and further investigate its specific mechanisms in the immune microenvironment.

5. Conclusion

This study integrates Mendelian randomization analysis and bioinformatics methods to explore the potential causal relationship between CD and PC, revealing the key role of the NOD2 gene in this process. NOD2 may promote the occurrence and progression of PC by regulating immune and inflammatory responses. Particularly, NOD2's role in the immune microenvironment of pancreatic cancer may provide new insights into immune evasion mechanisms. The expression of NOD2 is closely associated with the prognosis of pancreatic cancer patients, and its potential as a prognostic biomarker and therapeutic target for PC warrants further investigation. These findings provide new biological evidence for the link between CD and PC, and open new directions for future research on early diagnosis and immunotherapy for pancreatic cancer.

Disclosure statement

The author declares no conflict of interest.

References

[1] Bengtsson A, Andersson R, Ansari D, 2020, The Actual 5-Year Survivors of Pancreatic Ductal Adenocarcinoma Based on Real-World Data. Scientific Reports, 10(1): 16425.

[2] Fan Y, Wang M, Gao Y, et al., 2025, Genetic and Environmental Factors Influencing Crohn's Disease. World Journal of Gastrointestinal Surgery, 17(3): 98526.

[3] Jess T, Gamborg M, Matzen P, et al., 2005, Increased Risk of Intestinal Cancer in Crohn's Disease: A Meta-Analysis of Population-Based Cohort Studies. The American Journal of Gastroenterology, 100(12): 2724–2729.

[4] Bettenworth D, Bokemeyer A, Baker M, et al., 2019, Assessment of Crohn's Disease-Associated Small Bowel Strictures and Fibrosis on Cross-Sectional Imaging: A Systematic Review. Gut, 68(6): 1115–1126.

[5] Min Y, Liu Z, Li R, et al., 2023, Association Between Inflammatory Bowel Disease and Pancreatic Cancer: Results From the Two-Sample Mendelian Randomization Study. Frontiers in Oncology, 13: 1155123.

[6] Stalker J, Gibbins B, Meidl P, et al., 2004, The Ensembl Web Site: Mechanics of a Genome Browser. Genome Research, 14(5): 951–955.

[7] Tang Z, Kang B, Li C, et al., 2019, GEPIA2: An Enhanced Web Server for Large-Scale Expression Profiling and Interactive Analysis. Nucleic Acids Research, 47(W1): W556–W560.

[8] Zhou Y, Zhou B, Pache L, et al., 2019, Metascape Provides a Biologist-Oriented Resource for the Analysis of Systems-Level Datasets. Nature Communications, 10(1): 1523.

[9 Lánczky A, Győrffy B, 2021, Web-Based Survival Analysis Tool Tailored for Medical Research (KMplot): Development and Implementation. Journal of Medical Internet Research, 23(7): e27633.

[10] Thul P, Lindskog C, 2018, The Human Protein Atlas: A Spatial Map of the Human Proteome. Protein Science, 27(1): 233–244.

[11] Cui H, Zhao G, Lu Y, et al., 2025, TIMER3: An Enhanced Resource for Tumor Immune Analysis. Nucleic Acids Research, 53(W1): W534–W541.

[12] Gao J, Aksoy B, Dogrusoz U, et al., 2013, Integrative Analysis of Complex Cancer Genomics and Clinical Profiles Using the cBioPortal. Science Signaling, 6(269): p11.

[13] Masaki S, Masuta Y, Honjo H, et al., 2024, NOD2-Mediated Dual Negative Regulation of Inflammatory Responses Triggered by TLRs in the Gastrointestinal Tract. Frontiers in Immunology, 15: 1433620.

[14] Maeda S, Hsu L, Liu H, et al., 2005, Nod2 Mutation in Crohn's Disease Potentiates NF-κB Activity and IL-1β Processing. Science, 307(5710): 734–738.

[15] Cuthbert A, Fisher S, Mirza M, et al., 2002, The Contribution of NOD2 Gene Mutations to the Risk and Site of Disease in Inflammatory Bowel Disease. Gastroenterology, 122(4): 867–874.

[16] Branquinho D, Freire P, Sofia C, 2016, NOD2 Mutations and Colorectal Cancer – Where Do We Stand? World Journal of Gastrointestinal Surgery, 8(4): 284–293.

[17] Gurses S, Varghese N, Gupta D, 2024, Innate Immunity Gene Nod2 Protects Mice From Orthotopic Breast Cancer. Molecular Biology Reports, 51(1): 988.

[18] Wang Y, Miao Z, Qin X, et al., 2021, NOD2 Deficiency Confers a Pro-Tumorigenic Macrophage Phenotype to Promote Lung Adenocarcinoma Progression. Journal of Cellular and Molecular Medicine, 25(15): 7545–7558.