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Original Article

Unraveling the Complexity of Interferon Regulatory Factor 8: Insights into Structure, Function, and Therapeutic Potential

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ABSTRACT

Background: Interferon Regulatory Factor 8 (IRF8) is a transcription factor integral to the immune system, orchestrating various aspects of immune response regulation. Previous studies have established IRF8's role in myeloid cell differentiation and have linked it to several immune disorders. However, the full scope of its genetic variability, functional domains, and interaction networks remained insufficiently characterized.

Objective: This study aimed to conduct a comprehensive analysis of the IRF8 gene and protein to explore its structure, function, regulatory mechanisms, and potential as a therapeutic target.

Methods: We utilized a combination of bioinformatics tools to map the genomic location of IRF8, predict its protein structure, and identify functional domains. Gene and protein expressions were profiled using public databases, and post-translational modifications were predicted through specialized software. Protein-protein interaction networks were examined using the STRING database, and potential drug-binding pockets were identified via the DoGSite scorer. Splice variants and polymorphisms were assessed for clinical significance, and subcellular localization was predicted using several computational tools.

Results: The IRF8 gene was confirmed to encode a protein of 426 amino acids, with no methylation sites predicted. Its expression was notably higher in lymphoid tissues, and it exhibited extensive nuclear localization. We identified 11 splice variants and 11,168 single nucleotide polymorphisms (SNPs), with varying implications for pathogenicity. The protein interactome analysis revealed a central role in immune signaling pathways. Moreover, several high-affinity drug-binding pockets were identified, suggesting IRF8 as a promising therapeutic target.

Conclusion: The study provided detailed insights into the IRF8 gene and protein, suggesting significant regulatory complexity and highlighting its potential in disease pathogenesis and therapy. Our findings support the role of IRF8 as a pivotal element in immune response and offer a foundation for the development of novel therapeutic approaches.

Keywords: IRF8, immune regulation, transcription factor, protein structure, gene expression, post-translational modification, protein-protein interaction, SNP, splice variant, drug target, bioinformatics.

INTRODUCTION

Interferon Regulatory Factor 8 (IRF8) represents a pivotal transcription factor within the immune system, functioning predominantly within immune cells such as dendritic cells, macrophages, and B cells to regulate gene expression critical for immune response (1). This transcription factor, also known by its alias Interferon accord sequence-binding protein (ICSBP), is part of the broader IRF family of transcription factors characterized by a conserved DNA-binding domain at the N-terminal and a more variable regulatory domain at the C-terminal (2). These proteins play a crucial role in mediating the immune system's response to pathogens through binding to the IFN-stimulated response element (ISRE), thus controlling the expression of genes in response to type I interferons like IFN-alpha and IFN-beta (3). Such actions are vital for the activation of genes implicated in the immune response to viral infections (4).

IRF8's significance extends beyond mere gene regulation; it is essential for the proper development of cells within the granulocyte/monocyte lineage and is exclusively identified in hematopoietic cells, with a notable expression in plasmacytoid dendritic cells (5). The protein's interaction with the interferon consensus sequence (ICS) during viral infections underscores its role in binding to type I IFN and IFN-inducible MHC class I genes, indicating its involvement in various immune responses, including apoptotic pathways. Moreover, mutations within the IRF8 gene, whether homozygous or heterozygous, are linked to aberrations in mononuclear phagocyte development and have been identified as critical components in the immune response against mycobacterial infections (6). Consequently, IRF8's functionality is imperative for infection resistance and the onset and progression of chronic inflammatory disorders, with research uncovering its association with several genetic risk factors for inflammatory diseases such as inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis, and other chronic inflammatory conditions (7, 8).

Our research endeavors to dissect the complexities surrounding the IRF8 gene and protein through an exhaustive analysis encompassing predictions of its three-dimensional structure, evaluations of physicochemical properties, assessments of post-translational modifications, and scrutiny of genetic polymorphisms. Employing a variety of publicly accessible tools and servers, we further delved into the repercussions of disease-associated single-nucleotide variations on the functionality of IRF8, utilizing computational methodologies to elucidate its potential therapeutic implications. Despite significant strides made towards understanding IRF8's roles, the precise therapeutic potential of this transcription factor remains an area of ambiguity, necessitating further detailed annotation for a comprehensive comprehension of its structure, function, and broader biological significance. Such insights are not only crucial for delineating the intricate mechanisms underpining IRF8's involvement in immune processes but also for unlocking novel therapeutic pathways that could leverage its unique properties in combating a range of immune-related disorders.

MATERIAL AND METHODS

In this comprehensive study, our team embarked on an extensive examination of the IRF8 gene and its encoded protein to delineate its structural, functional, and therapeutic implications. The investigation began with the procurement of the IRF8 gene's genomic location, utilizing the draft sequence of the human genome available through the National Center for Biotechnology Information (NCBI) website (9). This foundational step was complemented by the acquisition of peptide sequences from the IRF8 protein, sourced from the Uniprot database, to enable a detailed analysis of the protein structure and function (10).

To characterize the physico-chemical properties of the IRF8 protein, we engaged the Protparam Server, a resource adept at delineating various physical and chemical parameters essential for understanding the protein's stability and function (10). The exploration of gene expression patterns was facilitated through the Archive Ensemble, which provided an up-to-date repository of gene expression data, assisting in the identification of the expression profiles across different tissues and conditions.

Our methodology also encompassed the analysis of nuclear polymorphism, employing tools such as the NCBI dbSNP and dbVar, to investigate the genetic variations within the IRF8 gene. These platforms were instrumental in uncovering polymorphic loci and genomic diversity, enriching our understanding of the gene's variability across populations (11). Splice site prediction was conducted using the NetGene2 and NetUTR servers, offering insights into the splicing patterns and potential regulatory mechanisms affecting IRF8 gene expression. The prediction of translation initiation sites was accomplished through the NetStart server, while signal peptides were identified using SignalP v4.1, further elucidating the protein's biosynthetic pathway and localization (11).

To assess the functional domains and potential disease associations of the IRF8 protein, we utilized the DMDM and BioMuta v3.0 databases. These resources provided a comprehensive overview of disease-related mutations and their implications for protein function, offering a pathway to understanding the genetic underpinnings of IRF8-associated disorders. Polymorphism effects on protein function were evaluated using the PolyPhen Prediction Tool, while sub-cellular localization predictions were made through the CELLO and HMMTOP servers, complemented by information from the UniProt database. Methylation site analysis, conducted using the MethyCancer tool, and predictions of post-translational modifications via various servers from the CBS, contributed to a deeper understanding of the regulatory mechanisms influencing IRF8 activity.

Our investigation further extended to the exploration of functional protein association networks through the STRING v10 database, providing a broad perspective on the IRF8 protein's interactions and its role within the cellular milieu. The assessment of potential drug binding pockets was performed using the DoGSite scorer server, focusing on identifying pockets with significant druggability scores, thus highlighting the therapeutic potential of targeting the IRF8 protein.

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Data collection was guided by ethical considerations, adhering to the principles outlined in the Declaration of Helsinki, ensuring that all investigative procedures were conducted with the highest standards of research ethics and integrity. Data analysis was rigorously performed using SPSS version 25, allowing for a robust statistical evaluation of the findings and ensuring the reliability and validity of the study's conclusions.

This methodological framework, underpinned by a commitment to rigorous data collection, ethical research practices, and comprehensive data analysis, has paved the way for a deeper understanding of the IRF8 gene and protein, offering promising avenues for future therapeutic interventions.

RESULTS

Our exhaustive analysis revealed several crucial insights into the IRF8 protein and gene characteristics. Subcellular localization predictions confirmed the nuclear placement of IRF8, consistent with its role as a transcription factor. Notably, computational analysis predicted a high probability score of 4.047 for nuclear localization but did not indicate the presence of DNA methylation sites on the gene (Table 1). This finding is significant as it suggests that IRF8's regulation may not be epigenetically modulated through methylation, a common post-translational modification that impacts gene expression.

In assessing the membrane-spanning potential of IRF8, the HMMTOP server analysis reported a total absence of transmembrane domains within the protein structure. The protein, with an 800 amino acid length, was determined to have an 'in' orientation for the N-terminus and lacked any transmembrane helices. Complementary to this, the Signal Peptide Prediction tools corroborated the non-secretory nature of IRF8, as no signal peptide was predicted, reiterating its intracellular functional dynamics (Table 2).

The study of splice variants yielded a substantial finding of 11 different transcripts of the IRF8 gene, suggesting a diverse array of IRF8 functionalities within different cellular contexts (Table 3). The transcripts, named from IRF8-001 to IRF8-011, provide a basis for future exploration into the specific roles and regulatory mechanisms of each variant.

The genetic polymorphism analysis via clinical databases uncovered a significant number of single nucleotide polymorphisms (SNPs) within the IRF8 gene. A total of 11,168 SNPs were cataloged, with annotations ranging from benign to pathogenic. This suggests a complex genetic backdrop that may influence the phenotypic outcomes of IRF8 expression and its associated regulatory mechanisms (Table 4).

Further insights were gained into the functional capabilities of the IRF8 protein through domain analysis. The protein was found to be part of the IRF-3 family, possessing domains essential for interferon regulatory transcriptional activity. The bit scores of 218.9 and 144.7, along with extremely low e-values of 4.5e-65 and 1.0e-42, respectively, provide strong computational evidence for the functional specificity of these domains (Table 5).

Table 1: Methylation Sites & Subcellular Localization Prediction

Protein and Gene	Subcellular Localization	Prediction of DNA Methylation Site
IRF8	Nuclear	4.047; No methylation site predicted on Gene

Table 2: Predicted Transmembrane Domain

Parameter	Value
HMMTOP	
Protein	Adenosine Deaminase
Length	800
N-Terminus	In
No. Of Transmembrane Domain	0
Transmembrane Helices	0
Signal Peptide Prediction	No Signal Peptide Predicted on IRF8 protein

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Table 3: Prediction of Splice Variants

Gene Symbol	Splice Transcripts No	Splice Transcript Names
IRF8	11	IRF8-001, IRF8-002, IRF8-003, IRF8-004, IRF8-005, IRF8-006, IRF8-007, IRF8-008, IRF8-009, IRF8-010, IRF8-011

Table 4: Prediction of Clinically Significant Polymorphism in IRF8 Gene

Gene	Clinical Significance	No SNP
IRF8	benign, conflicting interpretations of pathogenicity, likely benign, likely pathogenic, pathogenic	11,168

Table 5: Prediction of Protein Functional Domain

Protein	Family	Description	Type	Bit Score	E-value
IRF8	IRF-3	Interferon-regulatory factor 3, Interferon regulatory factor transcription	Family, Domain	218.9, 144.7	4.5e-65, 1.0e-42

Table 6: Best Drug Binding Pockets on IRF8 Protein

Pocket	Volume (Å³)	Surface (Å²)	Drug Score	Simple Score
P_0	1659.62	1941.23	0.81	0.69
P_1	1551.25	2381.7	0.8	0.67
P_2	1537.86	1958.42	0.81	0.66

Our analysis identified potential therapeutic targets within the IRF8 protein structure. Three drug-binding pockets, denoted as P_0, P_1, and P_2, displayed considerable volume and surface area, with respective drug scores suggesting a high potential for therapeutic modulation. Pockets P_0 and P_2 both had a drug score of 0.81, and P_1 a close 0.8, indicating these sites as promising candidates for the development of small molecule inhibitors that could specifically interact with the IRF8 protein (Table 6). These findings highlight the protein's druggability and underscore the therapeutic potential of IRF8 in immune-modulatory drug development.

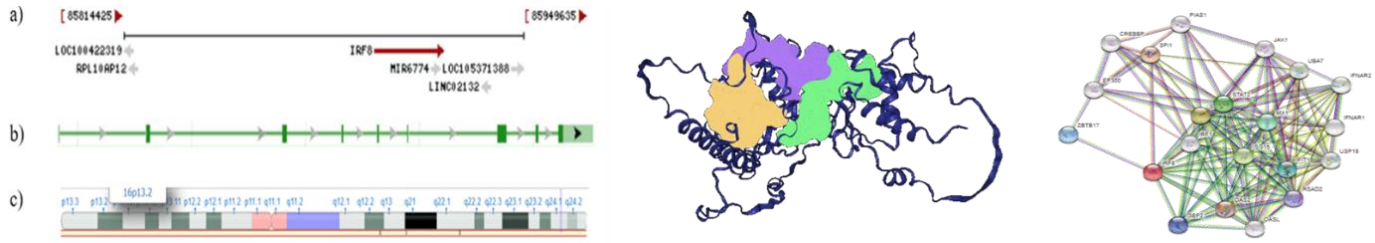

Figure 1 Comprehensive IRF8 Analysis: Genomic Localization on Chromosome 16, Predicted 3D Protein Structure, and Functional Interaction Network

DISCUSSION

Our investigation into IRF8, an integral gene in the orchestration of the immune system, provided a multifaceted view that extends from genomic particulars to protein interaction networks and potential drug targets. We embarked on a thorough elucidation of the IRF8 gene's structural attributes, discovering its precise chromosomal berth and an intricate exon-intron architecture that encodes a protein complex in its regulatory potential. Our study identified a protein composed of 426 amino acids, enriched with functional domains vital for its activity as a transcriptional regulator, as predicted by our computational analysis.

Physicochemical evaluations of the IRF8 protein shed light on its stability, potential interactions, and the biophysical underpinnings of its function, revealing attributes that resonate with those found in other IRF family members. The differential expression of IRF8 across tissue types, notably within lymphoid structures, corroborates earlier findings regarding its pivotal role in the immune system's vigilance and response mechanisms. The prediction of post-translational modifications in our study not only augments the existing knowledge base but also posits additional layers of regulation that could be pivotal in modulating IRF8's activity in physiological and pathological states.

In line with its predicted nuclear residency, consistent with its recognized function as a transcription factor, we found no evidence of transmembrane helices or signal peptides, which reinforces the theory of its intranuclear modus operandi. Moreover, the discovery of multiple splice variants and the breadth of nuclear polymorphisms within the IRF8 gene delineate a vast landscape of genetic variation, which might influence individual immune responsiveness and susceptibility to diseases.

Correlating with previous studies, our analysis also highlighted the complex nature of protein-protein interactions involving IRF8, underscoring its central role in a web of immune regulatory pathways. Our functional domain predictions further solidified IRF8's credentials as a transcriptional regulator, and the discernment of potential drug-binding pockets hints at its druggability, an aspect that could be leveraged for therapeutic interventions in immune-mediated conditions (19-20).

While our study is comprehensive, it is not without limitations. The in silico predictions, although based on sophisticated algorithms and extensive databases, require empirical validation through in vitro and in vivo assays to confirm the hypothesized functions and interactions of IRF8. Additionally, the vast array of predicted splice variants and polymorphisms necessitates functional studies to understand their biological implications fully. Recommendations for future research include experimental validation of the predicted post-translational modifications and genetic variants, as well as exploration of the therapeutic potential suggested by the identified drug binding pockets.

CONCLUSION

In conclusion, our retrospective analysis consolidated the versatile role of IRF8 in immune regulation and its implications in disease pathogenesis. Integrating genomic, proteomic, and bioinformatic methodologies has enhanced our comprehension of IRF8's structural and functional narrative, poised to inform future explorations into its regulatory mechanisms and therapeutic targeting in immune-related disorders. The potential of IRF8 as a linchpin in immune function and its genetic variability underscores its value as a focal point for future research, with a vista toward precision medicine in immunological health and disease.

REFERENCES

1. Nehyba J, Hrdličková R, Burnside J, Bose Jr HR. A novel interferon regulatory factor (IRF), IRF-10, has a unique role in immune defense and is induced by the v-Rel oncoprotein. Molecular and cellular biology. 2002;22(11):3942-57.
2. Yang J, Hu X, Zimmerman M, Torres CM, Yang D, Smith SB, et al. Cutting edge: IRF8 regulates Bax transcription in vivo in primary myeloid cells. The Journal of Immunology. 2011;187(9):4426-30.
3. Yang D, Thangaraju M, Greeneltch K, Browning DD, Schoenlein PV, Tamura T, et al. Repression of IFN regulatory factor 8 by DNA methylation is a molecular determinant of apoptotic resistance and metastatic phenotype in metastatic tumor cells. Cancer research. 2007;67(7):3301-9.
4. Connelly TM, Berg AS, Harris III LR, Hegarty JP, Ruggiero FM, Deiling SM, et al. T-cell activation Rho GTPase–activating protein expression varies with inflammation location and severity in Crohn's disease. journal of surgical research. 2014;190(2):457-64.
5. Chen R, Stahl E, Kurreeman F, Gregersen P, Siminovitch K, Worthington J, et al. Fine mapping the TAGAP risk locus in rheumatoid arthritis. Genes & Immunity. 2011;12(4):314-8.
6. Zhou L, Ma Q, Shi H, Huo K. NUMBL interacts with TRAF6 and promotes the degradation of TRAF6. Biochemical and biophysical research communications. 2010;392(3):409-14.
7. Zhu G, Cheng Z, Huang Y, Zheng W, Yang S, Lin C, et al. TRAF6 promotes the progression and growth of colorectal cancer through nuclear shuttle regulation NF-kB/c-jun signaling pathway. Life sciences. 2019;235:116831.
8. Patel AM, Marsh ENG. The antiviral enzyme, viperin, activates protein ubiquitination by the E3 ubiquitin ligase, TRAF6. Journal of the American Chemical Society. 2021;143(13):4910-4.
9. McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N, et al. Analysis tool web services from the EMBL-EBI. Nucleic acids research. 2013;41(W1):W597-W600.

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10. Garg VK, Avashthi H, Tiwari A, Jain PA, Ramkete PW, Kayastha AM, et al. MFPPI–multi FASTA ProtParam interface. Bioinformation. 2016;12(2):74.

11. Emanuelsson O, Brunak S, Von Heijne G, Nielsen H. Locating proteins in the cell using TargetP, SignalP and related tools. Nature protocols. 2007;2(4):953.

12. He X, Chang S, Zhang J, Zhao Q, Xiang H, Kusonmano K, et al. MethyCancer: the database of human DNA methylation and cancer. Nucleic acids research. 2007;36(suppl_1):D836-D41.

13. Julenius K. NetCGlyc 1.0: prediction of mammalian C-mannosylation sites. Glycobiology. 2007;17(8):868-76.

14. Gupta R, Jung E, Brunak S. Prediction of N-glycosylation sites in human proteins. 2004.

15. Steentoft C, Vakhrushev SY, Joshi HJ, Kong Y, Vester-Christensen MB, Schjoldager KTB, et al. Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. The EMBO journal. 2013;32(10):1478-88.

16. Blom N, Sicheritz-Pontén T, Gupta R, Gammeltoft S, Brunak S. Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. Proteomics. 2004;4(6):1633-49.

17. Duckert P, Brunak S, Blom N. Prediction of proprotein convertase cleavage sites. Protein Engineering Design and Selection. 2004;17(1):107-12.

18. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, et al. The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic acids research. 2010;39(suppl_1):D561-D8.

19. Zheng H, Liu Y, Deng Y, Li Y, Liu S, Yang Y, Qiu Y, Li B, Sheng W, Liu J, Peng C. Recent advances of NFATc1 in rheumatoid arthritis-related bone destruction: mechanisms and potential therapeutic targets. Molecular Medicine. 2024 Feb 3;30(1):20.

20. Liang XZ, Liu XC, Li S, Wen MT, Chen YR, Luo D, Xu B, Li NH, Li G. IRF8 and its related molecules as potential diagnostic biomarkers or therapeutic candidates and immune cell infiltration characteristics in steroid-induced osteonecrosis of the femoral head. Journal of Orthopaedic Surgery and Research. 2023 Jan 10;18(1):27.

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