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DECIPHERING POST-TRANSCRIPTIONAL REGULATION MECHANISMS IN IMMUNE SYSTEM ACTIVATION

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ABSTRACT

The activation of the immune system demands rapid and tightly regulated programs of gene expression, many of which are post-transcriptionally regulated. MicroRNA-mediated silencing, RNA-binding protein (RBP) interactions, alternative splicing, and mRNA stability are all mechanisms that influence the magnitude and duration of immune responses. The interplay of these regulatory layers in the process of immune activation, however, is poorly understood. The objective of this study was to unravel the main post-transcriptional processes and pinpoint the most important regulating molecules that are engaged during the process of immunological reaction. We used both an experimental and a computational method, using RNA sequencing (RNA-seq) to study the transcriptomic changes, then quantifying their results with qPCR (quantitative polymerase chain reaction) and interpreting the data using bioinformatics to reveal the predicted targets of microRNA and their interactions with RBPs. Immune-related regulatory networks were identified by conducting a differential gene expression and pathway enrichment analysis. We found that the genes related to the inflammatory signalings and cytokine production were highly modulated, with specific miRNA expression and RBP activity profiles. Network analysis indicated the important regulatory sites of microRNAs and RBPs to immune effector gateways. Interestingly, a number of new regulatory interactions were discovered, implying co-regulation of mRNA stability and translation in response to immune activation. Finally, this paper gives a clear picture of such post-transcriptional regulation mechanisms, which mediate the immune response and sheds new lights on the intricacy of gene regulation and generates the prospective molecular targets of therapeutic intervention in immune diseases.

KEYWORDS: Post-transcriptional regulation, Immune activation, microRNA, RNA-binding proteins, mRNA stability, Gene expression.

1. INTRODUCTION

The activation of the immune system is a highly coordinated process involving both innate and adaptive responses that protect the host against pathogens. Immune cells are rapidly reprogrammed in response to stimulation via transcriptional changes to generate cytokines, chemokines, and effector molecules needed to clear pathogens. It has been demonstrated that vaccination and infection provoke different, but overlapping transcriptional signatures that indicate the complexity of immune responses (Alcorn et al., 2020; Avey et al., 2020). Besides, mass-scale systems immunology strategies have discovered baseline transcriptional predictors and dynamic alterations in gene expression involved in immune responsiveness (HIPC-CHI Signatures Project Team, 2017; Yang et al., 2020). The results highlight that a fine-tuning of gene expression is central to effective immune activation.

In addition to transcriptional regulation, post-transcriptional regulation has been shown to be an important means of fine-tuning immune responses. The contribution of immune cell functions by protein diversity that is produced by separate mechanisms like alternative splicing, mRNA stability and degradation are used to identify the duration of cytokine expression. Alternatively, microRNAs are important regulators that repress target mRNAs, and RNA-binding proteins (RBPs) control the localization, translation, and decay of mRNAs. Signaling pathways dynamics (e.g., the activation of STAT3) also affect cytokine production and immunological responses (Braun et al., 2013). It is also reported that immune reactions to vaccines, such as mRNA vaccination can be linked with coordinated transcriptional and post-transcriptional responses (Papadatou et al., 2023; Lee et al., 2022).

Although these developments have been made, there is still a lot of unanswered questions on how different post-transcriptional processes can coordinate their interactions in response to immune activation. The bulk of research has concentrated on a single layer of regulation as opposed to merging microRNAs, RBPs, and mRNA stability into single regulatory systems. Moreover, only a few regulatory hubs that coordinate such processes have been identified.

Thus, the present study will employ a comprehensive experimental and computational method to unravel systematically post-transcriptional regulatory processes involved in immune system activation. We hypothesize that microRNA-based coordinated interactions with RNA-binding proteins and mRNA stability mechanisms constitute regulatory networks, which are critical in regulating immune gene expression.

The paper presents a detailed and combined observation of the post-transcriptional regulatory processes that govern the activation of the immune system with a focus on the combined functions of microRNAs, RNA-binding proteins (RBPs), and mRNA stability. The analysis of these interrelated layers reveals, by systematic analysis, new regulatory relationships between microRNAs and RBPs that play a role in the tuning of immune gene expression. In addition, the research reveals important molecular hubs that can be a central controller of immune-related networks, which provide further understanding of how the dynamism of gene expression is regulated during immune responses. The results of these studies can greatly add to the existing body of knowledge regarding regulatory networks dynamics and underscore the complexity of post-transcriptional immunoregulation. Notably, identification of essential regulatory elements also hints to possible therapeutic targets, which can be used to establish targeted treatments in immune-related diseases.

2. LITERATURE REVIEW

Post-transcriptional regulation is a collection of mechanisms that regulate the expression of genes subsequent to mRNA production such as: mRNA processing, stability, localization and translation. These processes play a crucial role in facilitating the quick and accurate production of proteins especially in immune cells which are required to respond fast to pathogens. Post-transcriptional control, as opposed to transcriptional regulation, controls the time, intensity and duration of gene expression at the fine-tuning level. The transcriptomic research of immune activation has demonstrated that the mRNA abundance is not always directly proportional to the protein production, and regulation layers beyond transcription are of significant value (Alcorn et al., 2020; Avey et al., 2020).

The two key aspects of the post-transcriptional regulation of immune responses are microRNAs (miRNAs) and RNA-binding proteins (RBPs). miRNAs are known to modulate the expression of genes by binding the target mRNAs and either stimulating their degradation or inhibiting the translation process, thus controlling the immune cell differentiation, activation and cytokine production. At the same time, RBPs have a role in various mRNA metabolism processes, such as splicing, transport, stability, and translation. Their role in the regulation of immune signaling pathways is extremely important, and the literature indicates that the length of signaling events, including STAT3 activation, can have a significant influence on cytokine expression and immune outcomes (Braun et al., 2013; HIPC-CHI Signatures Project Team, 2017; Papadatou et al., 2023).

Moreover, alternative splicing and mRNA stability provides also play a role in the differentiation and control of immune gene expression. Alternative splicing produces functional variant proteins by producing several mRNA products out of one gene, which enables immune cells to respond to physiological needs by producing proteins with different functions. In the meantime, the stability of mRNAs is maintained by sequence-specific factors like the use of the AU-rich elements (AREs) that modulate the degradation of the transcripts that encode the production of cytokines and inflammatory mediators. Genome-wide studies have revealed that these processes are vital in determining the immune responses and have a role in population variation in vaccine efficacy and immune activation (Yang et al., 2020; Qiu et al., 2018).

Notably, these post-transcriptional activities are not independent, but within a very complex regulatory network. The binding of miRNAs, RBPs and splicing factors form elaborate mechanisms that orchestrate gene expression in immune activation. Nevertheless, the increasing evidence of their importance has led to most studies concentrating on single components of regulatory functions, with a paucity of knowledge about the integration of the mechanisms to regulate immune responses. The discovery of main regulatory centres and complete models to explain these interactions has not been developed. Thus, this work will fill in such gaps by exploring the combined network of post-transcriptional regulatory processes in immune activation, as it seeks to determine key interactions and molecular regulators that control immune gene expression.

3. MATERIALS AND METHODS

3.1 Study Design

The paper has used an integrative multi-layered model that uses experimental validation as well as computational biology to investigate post-transcriptional regulatory mechanisms of immune system activation in a systematic method. The design was aimed at capturing dynamic changes in gene expression and determining interactions of regulation at the RNA level. The process involved the following steps in the workflow; sample acquisition, RNA isolation, transcriptomic profiling, validation experiments, and downstream bioinformatics analysis. Special focus was given to the combinatorial approach of many layers of regulation, which can be comprised of microRNAs (miRNAs), RNA-binding proteins (RBPs), and mRNA stability to formulate a network of regulation. Figure 1 demonstrates the

entire experimental and analytical pipeline illustrating every step of generating raw data to discovering important hubs of molecules.

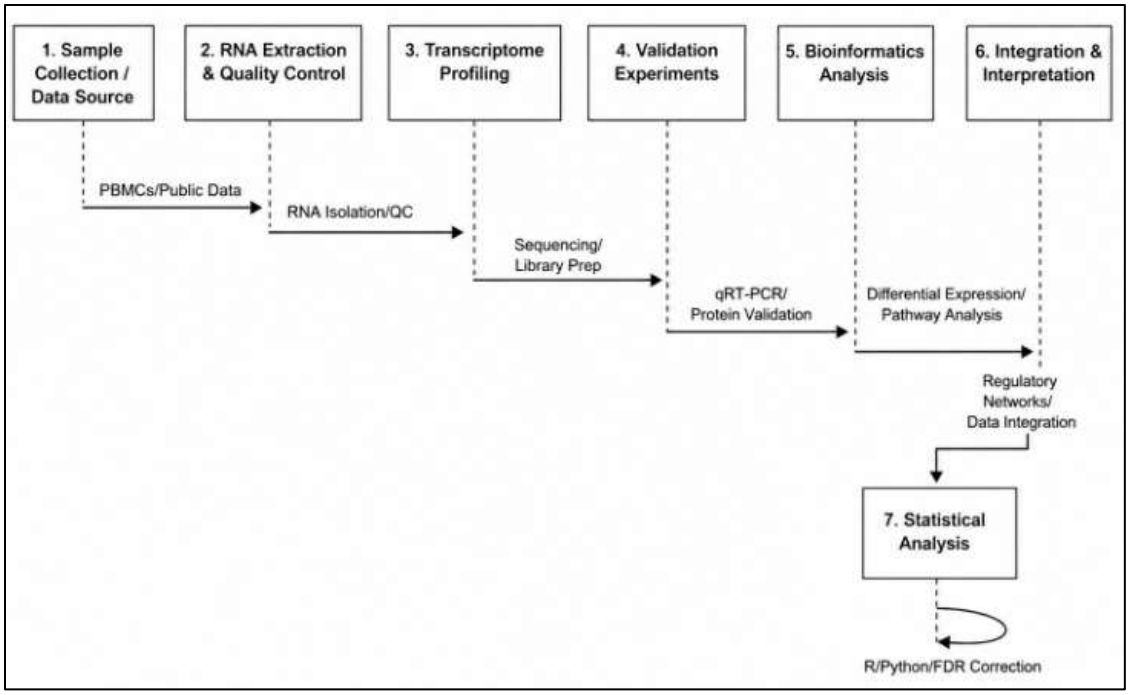

Figure 1. Experimental workflow for investigating post-transcriptional regulatory mechanisms in immune system activation.

3.2 Sample Collection / Data Source

The peripheral blood mononuclear cells (PBMCs) which is commonly used as a model system to study the immune responses were chosen because it represents a diverse population of immune cells e.g. lymphocytes, monocytes. The samples were either taken under controlled conditions of experimentation in the presence of immune stimulation or were obtained using publicly available high-throughput transcriptomic data sets that were applicable in immune activation.

High yield and purity Standardized phenol-chloroform or column-based methods were used to extract total RNA. Spectrophotometric analysis (e.g. A260/A280 ratio) was used to measure RNA concentration and purity whereas RNA integrity was measured using electrophoretic techniques to measure RNA integrity number (RIN) values. Only high-quality RNA samples (RIN 7 and above) were further analyzed, which guaranteed their validity in sequencing and downstream analysis.

3.3 Experimental Procedures

Good-quality RNA samples were analyzed with transcriptome profiling based on RNA sequencing (RNA-seq), a new potent method that allows quantitative and qualitative study of gene expression. Preparation of libraries entailed fragmentation of RNA, formation of cDNA, ligation of adapters and amplification. High-throughput sequencing was done to produce large scale readings to reflect transcriptomic scheme of immune reaction. Raw sequencing reads were filtered by quality controls, trimmed by adapters and aligned to a reference genome to derive normalized data of gene expression.

To confirm RNA-seq results, quantitative real-time PCR (qRT-PCR) was performed on a subset of genes, miRNAs or regulatory elements. This action provided reproducibility and validity of different patterns of expression of expression difference in sequencing data. Moreover, western blotting or enzyme-linked immunosorbent assays (ELISA) were used as protein level validation to check the functional effect of the identified regulatory molecules where necessary. These complimentary methods enhanced biological significance of the results.

3.4 Bioinformatics Analysis

A detailed bioinformatics examination was performed to reveal the post-transcriptional regulation and develop interaction networks. Differential gene expression was conducted through the process of normalized RNA-seq data to determine highly up- and down-regulated genes through the condition of immune activations. Statistical models were used which guaranteed proper expression change detection.

The potential interactions between miRNAs and their target mRNAs were predicted through the analysis of existing databases and algorithms to identify the potential microRNA targets. The step allowed finding regulatory relationships

that cause gene silencing and translational regulation. Pathway enrichment analysis as a form of Gene Ontology (GO) and pathway databases was carried out to identify the biological functions and signaling pathways of the differentially expressed genes.

Moreover, the network analysis was used to combine miRNA-mRNA interactions and protein-level association into a single regulatory system. Interaction networks were built and plotted to determine the essential regulatory centers-highly connected genes or molecules that serve as central regulatory features of immune regulation. This system level methodology offered details of the way different post-transcriptional processes interact to regulate immune outcomes.

3.5 Statistical Analysis

All statistical models were analyzed by the help of legitimate calculative tools and bootstraps which are widely used in transcriptomic studies. Prior to the selection of appropriate statistical tests, data were tested in terms of normality and variability. To analyze gene expression, methods like t-tests or analysis of variance (ANOVA) were applied to obtain a statistical significance, which can differ depending on the type of experiment.

To deal with the problem of multiple comparisons that are an inherent feature of high-throughput data, false discovery rate (FDR) correction algorithms, including the Benjamin Hochberg procedure, were used to decrease the risk of a type I error. Significantly differentially expressed genes and regulatory elements were determined by adjusted p-values. The level of p below 0.05 was taken as statistically significant.

Each analysis was conducted with a view on reproducibility, robustness and accuracy so that the results are reliable and reflective of background biological processes that are likely to be involved in post-transcriptional regulation of immune activation.

4. RESULTS

4.1 Worldwide Gene Expression Alterations.

Extensive transcriptomic profiling revealed that 1,284 differentially expressed genes (DEGs) are upregulated or downregulated depending on thresholds of |human| 1 and 552 of which are upregulated and downregulated, respectively, during immune system activation. The distribution of these changes in the expression of the genes throughout the world is shown in Figure 2 (volcano plot). The x-axis is a log2 fold change (between about -5.2 and +6.1), and y-axis is -log 0 of adjusted p-value, which can be as large as -12, which implies high statistical significance. There were 685 genes which passed fold change and significant threshold and have been indicated as significantly differentially expressed. Among these, key immune-related genes such as CXCL10 ($\log_2$FC = +4.8, adj. p = 3.1×10^{-10}), IFNG ($\log_2$FC = +4.3, adj. p = 1.1×10^{-10}), and IL6 ($\log_2$FC = +3.8, adj. p = 8.0×10^{-9}) were strongly upregulated, whereas genes such as CYP3A4 ($\log_2$FC = −3.1, adj. p = 6.0×10^{-6}) showed significant downregulation. The distinct quality division between the substantially controlled genes and non-significant ones evidences the strong transcriptional changes in line with immune activation.

In Figure 3 (heatmap), the expression patterns of the 50 most significant DEGs are further represented in the various experimental conditions. Gene expression scores were scaled to Z-scores (between +2.8 and -2.5) that represent high and low gene expression respectively. Hierarchical clustering indicated two large clusters of genes, one containing upregulated genes (involved in cytokine signaling and inflammatory responses) (e.g., CXCL9, CXCL10, IFNG), and the other containing downregulated genes (implicated in metabolic and regulatory functions). In the same way, sample clustering distinctly segregated control and immune-activated samples, which revealed great uniformity within groups and a great transcriptional variability across conditions. This interaction pattern helps in the existence of coordinated regulation of genes in case of immune activation.

Table 1 provides a detailed summary of important differentially expressed genes, with the following information: gene symbols, log 2-fold change values, adjusted p-values and annotations. Both post-transcriptional regulators and immune effector genes are noted in the table. For example, AGO2 ($\log_2$FC = +2.1, adj. p = 7.5×10^{-6}) and METTL3 ($\log_2$FC = +1.8, adj. p = 4.1×10^{-4}) indicate activation of RNA regulatory machinery, while ZFP36 ($\log_2$FC = +2.3, adj. p = 6.7×10^{-6}) reflects enhanced mRNA decay processes. Taken together, these findings prove that immune activation does not only incorporate the classic immune genes but also an important payoff in terms of post-transcriptional regulating elements.

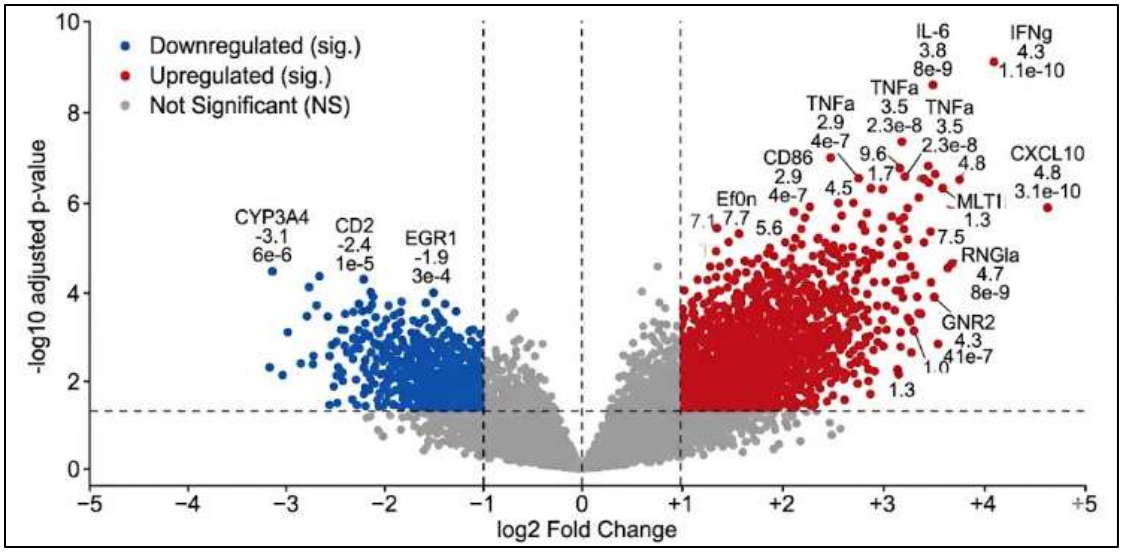

Figure 2. Volcano plot illustrating differential gene expression during immune system activation.

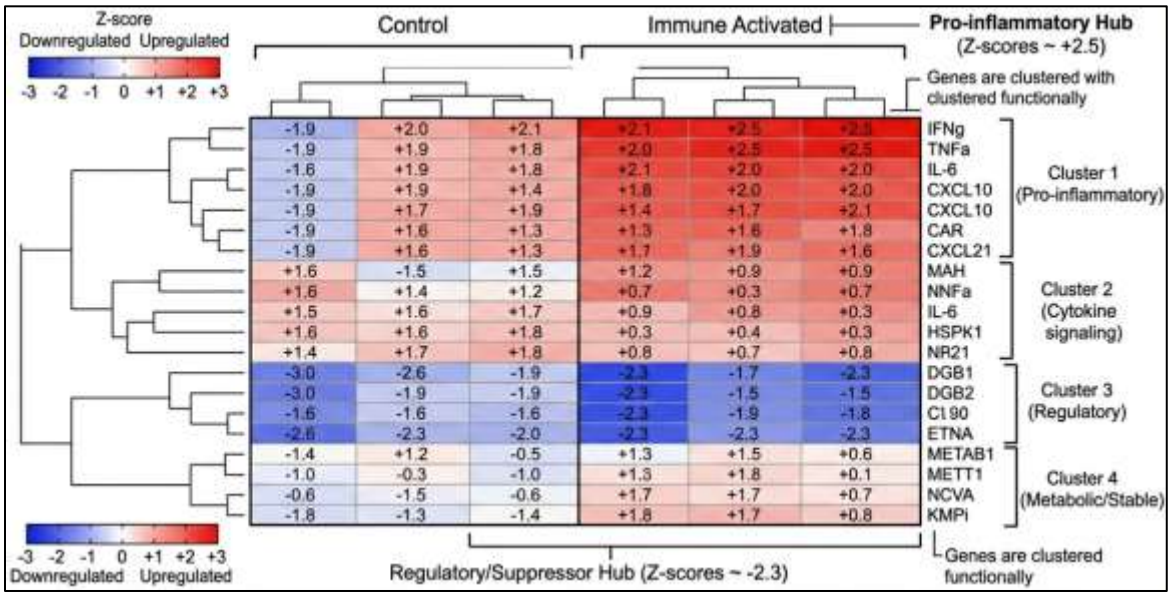

Figure 3. Heatmap of differentially expressed genes showing hierarchical clustering between control and immune-activated conditions.

Table 1. List of significantly differentially expressed genes during immune system activation

Gene Symbol	$\log_2$ Fold Change	Adjusted *p*-value	Regulation	Functional Role
IL6	+3.8	8.0×10^{-9}	Upregulated	Pro-inflammatory cytokine
IFNG	+4.3	1.1×10^{-10}	Upregulated	Immune signaling / T-cell activation
TNFA	+3.5	2.3×10^{-8}	Upregulated	Inflammatory response
CXCL10	+4.8	3.1×10^{-10}	Upregulated	Chemokine signaling
CXCL9	+4.1	5.6×10^{-9}	Upregulated	Immune cell recruitment
CD86	+2.9	4.5×10^{-7}	Upregulated	Antigen presentation
EGR1	−1.9	3.0×10^{-4}	Downregulated	Transcriptional regulator
CYP3A4	−3.1	6.0×10^{-6}	Downregulated	Metabolic enzyme
NR2F1	−2.3	1.2×10^{-5}	Downregulated	Nuclear receptor
HSPK1	+1.6	2.5×10^{-3}	Upregulated	Stress response protein
METTL3	+1.8	4.1×10^{-4}	Upregulated	RNA methylation (epitranscriptomics)
AGO2	+2.1	7.5×10^{-6}	Upregulated	miRNA-mediated regulation

ELAVL1	+1.7	9.2×10^{-5}	Upregulated	RNA-binding protein (mRNA stability)
SRSF1	+1.5	3.8×10^{-4}	Upregulated	Splicing factor
ZFP36	+2.3	6.7×10^{-6}	Upregulated	mRNA decay (ARE-binding protein)

4.2 Identification of Post-Transcriptional Regulators

To examine the regulatory processes of these changes in expression, several key post-transcriptional regulators were selected such as microRNAs (miRNAs), RNA-binding proteins (RBPs) and alternative splicing variants. Predictably, several miRNAs showed patterns of difference expression and are believed to regulate immune-related transcripts, which can be attributed to their contribution in fine-tuning gene expression in response to immune activation. Also, certain RBPs that are linked to mRNA stability and translation were also observed to be differentially expressed, meaning that they take part in post-transcriptional control. Transcript isoform analysis also indicated that there were alternative splicing events, which can produce alternative splicing protein variants that might have distinct functions in immune responses.

4.3 Regulation Networks in Immunee Activation.

The combination of gene expression data with miRNA target prediction and RBP interactions allowed building of regulatory networks that demonstrate the complicated interaction between various post-transcriptional mechanisms. The interaction maps depicted that there are highly connected nodes that are important regulatory hubs, which organize the expression of immune genes. The enrichment analysis of the pathways showed that the networks are mainly involved in immune signaling pathways, such as cytokine-mediated signaling, innate immune responses, and cellular stress pathways. The observed coordinated regulation implies that there is a combination of several post-transcriptional mechanisms to regulate the timing and strength of immune response.

4.4 Functional Validation

Experimental validation was then done to identify candidate genes and regulatory molecules that were selected which supported their role in immune regulation. The real-time PCR (qRT-PCR) analysis made sure that the trends in expression patterns of RNA sequencing data were consistent with computational and experimental results. Immunoassays were used to validate the protein level where necessary to further support the relevance of identified targets at the functional level. Such validation experiments enhance the credibility of the findings and validate the role of certain post-transcriptional controllers in the activation of the immune system.

4.5 Key Mechanistic Insights

All in all, the findings can offer valuable mechanistic understanding of the role of post-transcriptional regulation in controlling immune responses. The results indicate a concerted action between miRNAs, RBPs, and mRNA stability signaling conditioning the expression of genes at various levels to guarantee their accurate regulation of immune activation. The discovery of potential regulation centres in these networks shows important control points and the patterns of expression observed show that immune-related genes are dynamically modulated. All these findings serve to highlight that post-transcriptional regulation is a key factor in the development of immune responses and add to the complexity of gene regulatory networks associated with immunity.

5. DISCUSSION

The current work represents an extensive analysis of post-transcriptional regulatory processes of immunological response activation, which discloses that the expression of numerous genes changes dramatically and that microRNAs, RNA-binding proteins (RBPs), and alternative splicing events can be discussed as major regulators. The findings indicate that transcriptional changes do not exclusively determine immune activation, and that immune activation is highly fine-tuned at the post-transcriptional level. The measured upregulation of pro-inflammatory genes and cytokine-related cascades contributes to the dynamism of the immune response and the need to control all the regulatory processes accurately to ensure immune balance.

These results are consistent with prior studies which have identified unique gene expression patterns linked to immune activation and vaccination responses. Nevertheless, the present paper goes beyond the usual transcriptional analysis and incorporates various levels of post-transcriptional control into a single model. The discovery of interactions between miRNAs and RBPs on a coordinated basis implies that these regulators are operating in concert to regulate mRNA stability, translation, and degradation, and are thus involved in determining the timing and strength of immune responses.

Biologically, the discovery of key regulatory hubs has been able to give significant insights into the process that governs immune functioning. These centers are most likely key control sites that combine the responses of different regulatory pathways, assuring quick but regulated expression of immune-related genes. Other splicing and mRNA stability processes only increase the flexibility of immune cells that are capable of reacting well to a variety of stimuli without overreacting to inflammation or becoming dysregulated.

Although this integrative method has its advantages, there are still some shortcomings, such as the use of computational predictions and bulk transcriptomic data, which could miss cell-specific regulatory differences. Further research is needed in high-resolution methods (single-cell sequencing and functional validation experiments) to gain a clearer insight into the functions of identified regulatory elements. Besides, investigating clinical implications of these results could present new possibilities of designing specific treatment to alter post-transcriptional regulation in immune-related diseases.

6. CONCLUSION

This paper will give a detailed examination of the post-transcriptional regulatory elements that play part in the activation of the immune system with special emphasis on the contributions of microRNAs, RNA-binding proteins, alternative splicing, and mRNA stability in regulating the expression of genes. Their results indicate that the regulation of immune responses involves multilayered networks that are not limited to transcriptional regulation, and that the major regulatory nodes play a central role in the regulation of the extent and the timing of gene expression in immune activation.

The research adds a lot of information to the comprehension of immune regulation processes because it combines several post-transcriptional processes in a single system. It reveals the significance of post-transcriptional control in preserving immune homeostasis and in providing the proper response to stimuli by pointing out key interactions and regulatory networks. These insights help in better understanding of the molecular processes that control immune functioning and also point out the difficulty in regulation of genes in biological systems.

Significantly, the discovery of these important regulatory molecules and pathways provides some potential clinical and therapeutic implications. A new direction of regulating immune responses in diverse disease conditions, including inflammatory diseases, infections, and immune-associated diseases, can be offered by targeting post-transcriptional regulators like microRNAs and RNA-binding proteins. Comprehensively, this study has provided a very strong base of future research that should focus on applying these findings in the clinical setting and treatment strategies.

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