Research Article

Bioinformatics-Assisted Identification of Therapeutic Targets and Nanoparticle-Based Delivery of Mrna Vaccines For Melanoma Immunotherapy

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Abstract

Melanoma is an aggressive form of skin cancer characterized by high metastatic potential, tumor heterogeneity, and resistance to conventional therapies. The emergence of immunotherapy has significantly improved treatment outcomes; however, the identification of effective therapeutic targets and efficient vaccine delivery systems remains a major challenge. The present study aimed to integrate bioinformatics-assisted target discovery with nanoparticle-based mRNA vaccine technology for melanoma immunotherapy. Melanoma transcriptomic and clinical datasets were obtained from public repositories and analyzed to identify differentially expressed genes, enriched biological pathways, hub genes, and potential therapeutic targets. Functional enrichment and protein–protein interaction analyses were performed to prioritize candidate genes associated with melanoma progression and immune regulation. Subsequently, immunoinformatics approaches were employed to identify and validate antigenic and immunogenic neoantigen candidates suitable for mRNA vaccine development. The selected antigens were incorporated into an optimized mRNA vaccine construct, which was evaluated for codon adaptation, GC content, structural stability, and immune response potential through computational analyses. To overcome the limitations associated with mRNA instability and poor cellular uptake, nanoparticle-based delivery systems were investigated for efficient vaccine encapsulation and intracellular transport. The results demonstrated the identification of promising melanoma-associated targets, favorable immunogenic properties of selected antigens, and enhanced delivery performance of nanoparticle formulations. Collectively, the findings suggest that the integration of bioinformatics, immunoinformatics, and nanotechnology provides a promising framework for the development of personalized mRNA vaccines and next-generation melanoma immunotherapies. Further experimental validation is warranted to support clinical translation.

Keywords: Melanoma; mRNA vaccine; Bioinformatics; Nanoparticles; Immunotherapy; Neoantigens.

1. Introduction

Melanoma is one of the most aggressive and lethal forms of skin cancer, originating from the malignant transformation of melanocytes, the pigment-producing cells responsible for skin coloration. Although melanoma accounts for a relatively small proportion of all skin cancer cases, it is responsible for the majority of skin cancer-related deaths due to its high metastatic potential and resistance to conventional therapies in advanced stages. The global incidence of melanoma has increased steadily over recent decades, particularly among fair-skinned populations exposed to excessive ultraviolet (UV) radiation (Idris et al., 2025). Environmental factors, genetic predisposition, and immune dysregulation collectively contribute to melanoma development and progression. Despite significant advances in diagnosis and treatment, advanced melanoma continues to present substantial clinical challenges, including tumor heterogeneity, immune evasion, treatment resistance, and disease recurrence. These challenges underscore the need for innovative therapeutic strategies capable of providing durable and personalized anti-tumor responses (Bertolotto, 2013).

The emergence of cancer immunotherapy has transformed the therapeutic landscape of melanoma. Unlike traditional treatment modalities such as surgery, chemotherapy, and radiotherapy, immunotherapy harnesses the body's immune system to recognize and eliminate malignant cells. The introduction of immune checkpoint inhibitors targeting programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-

lymphocyte-associated protein 4 (CTLA-4) has significantly improved survival outcomes in patients with advanced melanoma (Xiong et al., 2024). However, a considerable proportion of patients fail to respond to these therapies or eventually develop resistance. Furthermore, immune-related adverse events and the inability to predict treatment response remain major limitations. Consequently, there is growing interest in developing personalized immunotherapeutic approaches that can selectively target tumor-specific antigens while minimizing off-target effects (Bandara & Raveendran, 2025).

Among emerging cancer immunotherapies, messenger RNA (mRNA)-based vaccines have attracted considerable attention due to their versatility, safety, and ability to induce potent cellular and humoral immune responses. The remarkable success of mRNA vaccine technology during the coronavirus disease 2019 (COVID-19) pandemic has accelerated research into its application in oncology. Unlike conventional vaccines that rely on attenuated pathogens or protein antigens, mRNA vaccines deliver genetic instructions encoding specific antigens directly into host cells, enabling endogenous antigen production and presentation (Miao et al., 2021). This mechanism effectively stimulates both CD4+ helper T cells and CD8+ cytotoxic T lymphocytes, which play critical roles in anti-tumor immunity. Additionally, mRNA vaccines are non-integrating, transiently expressed, and can be rapidly designed and manufactured, making them particularly suitable for personalized cancer treatment. In melanoma, mRNA vaccines offer the opportunity to target tumor-associated antigens and neoantigens that are uniquely expressed by malignant cells, thereby enhancing therapeutic specificity and reducing the risk of autoimmune toxicity (Duan et al., 2022).

The identification of appropriate therapeutic targets is a critical prerequisite for successful cancer vaccine development. Advances in high-throughput sequencing technologies and computational biology have revolutionized the discovery of cancer-associated biomarkers and antigens. Bioinformatics has emerged as a powerful interdisciplinary field that integrates biological data with computational tools to analyze complex genomic, transcriptomic, proteomic, and immunological datasets. Through comprehensive analysis of large-scale public databases such as The Cancer Genome Atlas (TCGA), Gene Expression Omnibus (GEO), and other cancer repositories, researchers can identify differentially expressed genes, mutation profiles, signaling pathways, and immune-related biomarkers associated with melanoma progression. These datasets provide valuable insights into tumor biology and facilitate the discovery of candidate therapeutic targets with diagnostic, prognostic, and therapeutic significance (Jamalinia & Weiskirchen, 2025).

One of the most promising applications of bioinformatics in cancer immunotherapy is the identification of tumor-specific neoantigens. Neoantigens arise from somatic mutations within cancer cells and are absent in normal tissues, making them highly attractive targets for immune-based therapies. Computational algorithms can predict major histocompatibility complex (MHC) binding affinity, antigen processing, immunogenicity, and population coverage, enabling the prioritization of neoantigens with the highest therapeutic potential. Furthermore, integrative bioinformatics approaches such as differential gene expression analysis, protein–protein interaction network construction, pathway enrichment analysis, and immune infiltration assessment can reveal key molecular drivers of melanoma and identify novel vaccine targets. The combination of multi-omics analyses and machine learning techniques has further enhanced the accuracy and efficiency of therapeutic target discovery, paving the way for precision oncology (Okada et al., 2022; Xie et al., 2023).

Despite the significant promise of mRNA vaccines, several biological and technological challenges limit their clinical effectiveness. Naked mRNA molecules are highly susceptible to enzymatic degradation by extracellular ribonucleases and exhibit poor cellular uptake due to their large size and negative charge. Additionally, efficient delivery to antigen-presenting cells, particularly dendritic cells, remains a major obstacle. Following cellular internalization, mRNA must escape endosomal compartments to reach the cytoplasm, where translation occurs. Failure to overcome these barriers can significantly reduce antigen expression and immune activation. Therefore, the development of effective delivery systems is essential for realizing the full therapeutic potential of mRNA-based cancer vaccines (Wadhwa et al., 2020).

Nanotechnology has emerged as a transformative solution for enhancing the stability, delivery, and efficacy of mRNA therapeutics. Nanoparticle-based delivery systems protect mRNA from degradation, facilitate cellular uptake, improve biodistribution, and promote efficient antigen expression. Among various nanocarriers, lipid nanoparticles (LNPs) have gained particular prominence due to their successful application in clinically approved mRNA vaccines. LNPs encapsulate mRNA within lipid bilayers, shielding it from enzymatic degradation while enabling targeted delivery to immune cells (Zhang et al., 2024). Other nanoparticle platforms, including polymeric nanoparticles, lipid-polymer hybrid nanoparticles, inorganic nanoparticles, and biomimetic nanocarriers, have also

demonstrated promising results in preclinical cancer immunotherapy studies. These delivery systems can be engineered to incorporate targeting ligands, immune adjuvants, and controlled-release mechanisms, thereby enhancing vaccine potency and specificity (Chen et al., 2023).

In melanoma immunotherapy, the integration of bioinformatics-driven target identification with nanoparticle-mediated mRNA delivery represents a highly promising strategy for personalized treatment. Bioinformatics enables the systematic discovery and prioritization of melanoma-associated antigens and neoantigens, while advanced nanocarriers ensure their efficient delivery and expression within immune cells. This synergistic approach has the potential to generate robust anti-tumor immune responses capable of overcoming tumor heterogeneity and immune escape mechanisms. Moreover, nanoparticle-based mRNA vaccines can be combined with existing immunotherapeutic modalities, including immune checkpoint inhibitors and adoptive cell therapies, to further enhance therapeutic efficacy. As advances in computational biology, genomics, and nanotechnology continue to evolve, the development of personalized mRNA vaccine platforms is expected to play an increasingly important role in the future management of melanoma (Tague et al., 2025; Zoroddu & Bagella, 2025).

Therefore, the present study focuses on the bioinformatics-assisted identification of therapeutic targets for melanoma and the development of nanoparticle-based mRNA vaccine delivery strategies. By integrating genomic and transcriptomic analyses with advanced nanotechnology, this approach aims to identify clinically relevant antigens, optimize vaccine design, and enhance immune-mediated tumor eradication. Such a multidisciplinary framework may contribute to the development of next-generation precision immunotherapies capable of improving clinical outcomes for patients with melanoma while advancing the broader field of cancer vaccine research (Zhang et al., 2025).

2. Nanoparticle-Based Delivery of mRNA Vaccines

2.1 Challenges in mRNA Delivery

Messenger RNA (mRNA) vaccines have emerged as a promising platform for cancer immunotherapy due to their ability to induce potent antigen-specific immune responses. However, the successful delivery of mRNA remains a major challenge. One of the primary obstacles is the inherent instability of mRNA molecules. Because mRNA is highly susceptible to degradation by extracellular and intracellular ribonucleases (RNases), naked mRNA exhibits a short biological half-life and limited therapeutic efficacy. Consequently, protective delivery systems are required to preserve mRNA integrity during circulation and transport (Kong et al., 2023).

Another significant challenge is cellular uptake. The large molecular size and negatively charged phosphate backbone of mRNA prevent efficient passage across the cell membrane. As a result, naked mRNA is poorly internalized by target cells, leading to low levels of antigen expression. Efficient delivery vehicles are therefore necessary to facilitate cellular entry and ensure sufficient intracellular concentrations of mRNA (Miao et al., 2021).

Following cellular internalization, endosomal entrapment presents an additional barrier. Most delivery systems enter cells through endocytosis, resulting in the sequestration of mRNA within endosomes. If mRNA fails to escape these compartments before lysosomal degradation occurs, its therapeutic potential is significantly reduced. Therefore, strategies that enhance endosomal escape are critical for maximizing antigen production and immune activation in mRNA vaccine applications (Chehelgerdi & Chehelgerdi, 2023).

2.2 Nanocarrier Systems for mRNA Vaccine Delivery

Nanocarrier-based delivery systems have become indispensable for improving the stability, biodistribution, and intracellular delivery of mRNA vaccines. Among the various platforms, lipid nanoparticles (LNPs) are the most extensively studied and clinically validated. LNPs typically consist of ionizable lipids, phospholipids, cholesterol, and polyethylene glycol (PEG)-modified lipids. These components collectively protect mRNA from enzymatic degradation, facilitate cellular uptake, and promote endosomal escape. Upon administration, LNPs efficiently deliver mRNA into the cytoplasm, where it is translated into antigenic proteins capable of stimulating immune responses (Karmacharya et al., 2022). Polymeric nanoparticles represent another important class of delivery vehicles. These systems are commonly fabricated using biodegradable polymers such as poly(lactic-co-glycolic acid) (PLGA), chitosan, and polyethyleneimine (PEI). Polymeric nanoparticles offer advantages including controlled release, enhanced stability, and tunable physicochemical properties. Their ability to provide sustained antigen expression makes them attractive candidates for cancer vaccine applications. Furthermore, polymeric carriers can be engineered to improve targeting efficiency and reduce systemic toxicity (Bandyopadhyay et al., 2023).

Lipid–polymer hybrid nanoparticles combine the beneficial properties of both lipid and polymer-based systems. Typically, these nanoparticles consist of a polymeric core surrounded by a lipid shell. This hybrid architecture provides enhanced structural stability, efficient mRNA encapsulation, and improved cellular uptake. In addition, hybrid nanoparticles can be functionalized with targeting ligands to achieve selective delivery to immune cells or tumor tissues. Recent advances in nanotechnology have led to the development of multifunctional hybrid systems capable of co-delivering mRNA, adjuvants, and immune-modulating agents (Rawal & Patel, 2019). Each nanocarrier platform possesses distinct advantages and limitations. While LNPs demonstrate high transfection efficiency and clinical success, concerns related to storage stability and immune reactions remain. Polymeric nanoparticles offer superior stability and controlled release but may exhibit lower transfection efficiency. Hybrid nanoparticles seek to overcome these limitations by integrating the strengths of both systems. Collectively, these nanocarriers have significantly expanded the therapeutic potential of mRNA vaccines in cancer immunotherapy (Yu et al., 2026).

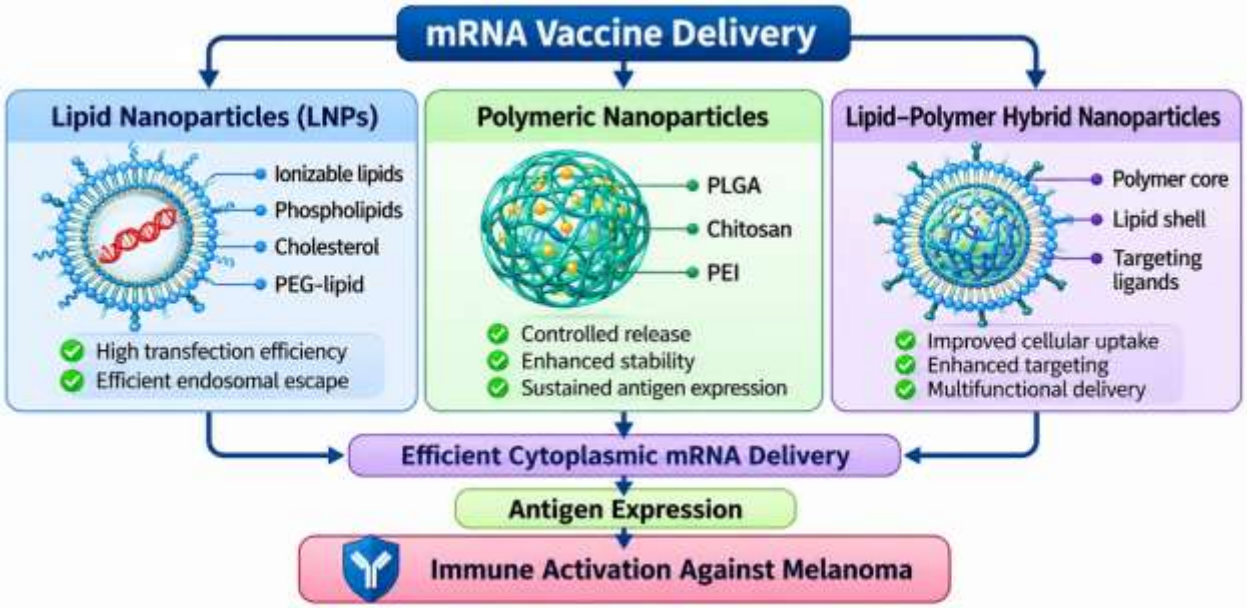


Figure 1: Major Nanocarrier Systems for mRNA Vaccine Delivery

2.3 Targeted Nanoparticle Delivery Strategies

Targeted delivery approaches have been developed to enhance the specificity and efficacy of nanoparticle-based mRNA vaccines. One of the most important strategies involves targeting dendritic cells, which are professional antigen-presenting cells responsible for initiating adaptive immune responses. By directing nanoparticles toward dendritic cells, vaccine antigens can be efficiently processed and presented to T lymphocytes, resulting in robust anti-tumor immunity (Lin et al., 2024).

Targeting the tumor microenvironment is another promising approach. The melanoma microenvironment contains various immunosuppressive factors that facilitate tumor progression and immune evasion. Nanoparticles can be engineered to selectively accumulate within tumor tissues through passive or active targeting mechanisms, thereby increasing local antigen expression and immune activation while minimizing systemic side effects (Jacob et al., 2024). Surface modification techniques further enhance nanoparticle performance. The incorporation of targeting ligands, antibodies, peptides, aptamers, or carbohydrate molecules onto nanoparticle surfaces can improve cellular recognition and uptake. Such modifications allow selective interaction with specific receptors expressed on immune cells or tumor cells, thereby improving therapeutic precision and reducing off-target effects (Adebanjo et al., 2025).

2.4 Clinical and Translational Perspectives

The clinical success of mRNA vaccine technology has accelerated its application in cancer treatment, including melanoma immunotherapy. Several clinical studies are currently evaluating nanoparticle-based mRNA vaccines either as monotherapies or in combination with immune checkpoint inhibitors. Early findings have demonstrated encouraging safety profiles, antigen-specific immune responses, and potential therapeutic benefits in patients with advanced malignancies (Yang et al., 2025).

Despite these advances, safety considerations remain important. Potential concerns include inflammatory responses, immunogenicity of delivery materials, and long-term biocompatibility. Regulatory challenges related to large-scale manufacturing, quality control, storage conditions, and product consistency must also be addressed before widespread clinical implementation. Future research is expected to focus on the development of next-generation nanocarriers with enhanced targeting capabilities, improved endosomal escape efficiency, and personalized antigen delivery. The integration of bioinformatics-driven antigen discovery with advanced nanoparticle engineering may facilitate the creation of highly individualized mRNA vaccines tailored to the molecular characteristics of each patient's melanoma. Such innovations have the potential to significantly improve treatment outcomes and establish mRNA nanovaccines as a cornerstone of precision cancer immunotherapy (Raza et al., 2025).

3. Materials and Methods

3.1 Data Collection and Preprocessing

Melanoma-related gene expression and clinical datasets were obtained from publicly accessible cancer databases, including The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO). TCGA-SKCM (Skin Cutaneous Melanoma) datasets were used to acquire transcriptomic profiles and corresponding clinical information, while relevant GEO datasets were selected to validate gene expression patterns and improve the robustness of the analysis. Data were downloaded in their processed formats following database guidelines and inclusion criteria. Quality control procedures were performed to identify incomplete records, duplicate samples, and outlier observations. Samples with excessive missing values or poor-quality expression profiles were excluded from subsequent analyses. Gene expression data were normalized using standard bioinformatics preprocessing methods to reduce technical variability and ensure comparability across samples. Batch effects originating from different experimental platforms were corrected when necessary. Clinical information, including patient age, gender, tumor stage, survival status, and follow-up duration, was extracted from the corresponding database records. The processed molecular and clinical datasets were integrated to facilitate downstream analyses, including differential gene expression analysis, biomarker identification, pathway enrichment studies, and therapeutic target discovery. All datasets utilized in this study were obtained from publicly available repositories and were analyzed in accordance with their respective data usage policies.

3.2 Identification of Differentially Expressed Genes (DEGs)

Differentially expressed genes (DEGs) between melanoma and normal skin tissue samples were identified using transcriptomic datasets obtained from TCGA and GEO repositories. Statistical analysis of gene expression profiles was performed using the R programming environment and Bioconductor packages. Raw expression data were first normalized to minimize technical variations and improve comparability among samples. Subsequently, expression levels of genes in melanoma tissues were compared with those in normal control samples using appropriate statistical methods, including moderated t-tests and linear modeling approaches. To control false-positive results arising from multiple comparisons, the Benjamini–Hochberg false discovery rate (FDR) correction method was applied (Dong et al., 2022).

Genes exhibiting significant differences in expression were selected based on predefined screening criteria. Specifically, genes with an adjusted p-value (FDR) of less than 0.05 and an absolute log2 fold change (|log2FC|) greater than 1.0 were considered statistically significant DEGs. Genes showing positive fold-change values were categorized as upregulated, whereas those with negative fold-change values were classified as downregulated. To facilitate interpretation of the results, visualization analyses were performed using volcano plots and heatmaps. Volcano plots were generated to display the distribution of significantly upregulated and downregulated genes based on fold-change and statistical significance. Heatmaps were constructed to illustrate the expression patterns of the most significant DEGs across melanoma and normal tissue samples, enabling the identification of distinct gene expression signatures associated with melanoma progression (Li & Xu, 2025).

3.3 Functional Enrichment and Pathway Analysis

To explore the biological significance of the identified differentially expressed genes (DEGs), functional enrichment and pathway analyses were performed. Gene Ontology (GO) analysis was conducted to categorize DEGs into three major functional domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). This

analysis facilitated the identification of biological activities, cellular localizations, and molecular functions significantly associated with melanoma development and progression (Long et al., 2019).

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was subsequently performed to identify signaling pathways and molecular networks involving the selected DEGs. Significantly enriched pathways were determined using adjusted p-values (FDR < 0.05), enabling the recognition of key biological mechanisms underlying melanoma pathogenesis. Particular attention was given to pathways associated with cell proliferation, apoptosis, immune regulation, tumor invasion, and metastatic progression (Tian et al., 2023). The enriched GO terms and KEGG pathways were interpreted to understand the functional roles of the identified genes in melanoma biology. These analyses provided insights into dysregulated molecular processes and helped prioritize potential therapeutic targets and vaccine candidate antigens for subsequent bioinformatics and immunological investigations (Guo et al., 2019).

3.4 Therapeutic Target and Neoantigen Identification

To identify potential therapeutic targets associated with melanoma, a protein–protein interaction (PPI) network was constructed using the differentially expressed genes (DEGs). Interaction data were obtained from established protein interaction databases, and the resulting network was visualized using Cytoscape software. The PPI network enabled the assessment of functional relationships among proteins and facilitated the identification of key molecular regulators involved in melanoma progression (Huang et al., 2022).

Hub genes were selected based on topological parameters, including degree centrality, betweenness centrality, and network connectivity. Genes exhibiting the highest interaction scores were considered hub genes and prioritized as potential therapeutic targets due to their critical roles in tumor growth, survival, and immune regulation. To identify vaccine candidates, immunogenic antigens and neoantigens were predicted using computational immunoinformatics approaches. Candidate genes were evaluated for antigenicity, expression levels, mutation frequency, and immune relevance. Predicted neoantigens were further screened for major histocompatibility complex (MHC) binding affinity and immunogenic potential. Highly immunogenic candidates were selected for subsequent mRNA vaccine design and nanoparticle-based delivery studies (Liang, 2008).

3.5 mRNA Vaccine Design and In Silico Evaluation

Selected melanoma-associated antigens and neoantigens were utilized for the design of the mRNA vaccine construct. Antigen sequences demonstrating high immunogenic potential and tumor specificity were prioritized. Codon optimization was performed to enhance translational efficiency and protein expression in human host cells while maintaining the original amino acid sequence. The optimized mRNA construct was subsequently evaluated using various immunoinformatics tools. Antigenicity assessment was conducted to determine the vaccine's ability to induce immune responses, whereas immunogenicity prediction evaluated its potential to activate T-cell-mediated immunity. Allergenicity analysis was performed to ensure minimal risk of allergic reactions, and toxicity prediction was carried out to confirm the safety profile of the selected vaccine candidates. Only constructs meeting all predefined criteria were considered suitable for further investigation (Liu et al., 2021).

3.6 Nanoparticle Formulation and Characterization

The optimized mRNA vaccine construct was encapsulated within nanoparticle-based delivery systems to enhance stability and cellular uptake. Nanoparticle formulations were prepared using established nanoencapsulation techniques, ensuring efficient loading and protection of the mRNA molecules from enzymatic degradation. The physicochemical properties of the formulated nanoparticles were subsequently characterized (Mehta et al., 2023).

Particle size distribution and polydispersity index were determined using dynamic light scattering (DLS), while zeta potential measurements were performed to evaluate surface charge and colloidal stability. Encapsulation efficiency was calculated by quantifying the amount of mRNA successfully incorporated within the nanoparticles relative to the initial loading concentration. The stability of the nanoparticle-encapsulated mRNA vaccine was assessed under different storage conditions. In addition, in vitro release studies were conducted to evaluate the release kinetics of mRNA from the nanoparticles over time, providing insights into their suitability for sustained and effective vaccine delivery (Maeyouf et al., 2023).

4. Results

4.1 Identification of Differentially Expressed Genes in Melanoma

Differential gene expression analysis revealed substantial transcriptional alterations between melanoma and normal skin tissues. A total of 1,248 genes were identified as significantly differentially expressed, including both upregulated and downregulated genes based on the predefined screening criteria (adjusted p-value < 0.05 and |log2FC| > 1). Volcano plot visualization demonstrated a clear separation between significantly altered and non-significant genes, highlighting the molecular heterogeneity of melanoma. Heatmap analysis further confirmed distinct expression patterns among samples and enabled the identification of highly dysregulated genes. Several candidate target genes exhibiting high fold-change values and strong statistical significance were prioritized for subsequent protein–protein interaction network analysis, neoantigen prediction, and mRNA vaccine development studies.

Table 1: Expression Profiles and Statistical Characteristics of Selected Differentially Expressed Genes in Melanoma

Gene Symbol	Expression Value	Fold Change	Significance Score	Network Score
GENE-1	312 ± 8.4	145 ± 3.2	87 ± 2.1	56 ± 1.7
GENE-2	298 ± 7.9	162 ± 4.1	94 ± 2.4	61 ± 1.9
GENE-3	335 ± 9.1	178 ± 4.5	102 ± 2.8	69 ± 2.1
GENE-4	356 ± 10.2	185 ± 4.8	111 ± 3.1	74 ± 2.3
GENE-5	389 ± 11.4	201 ± 5.3	126 ± 3.5	82 ± 2.6

Values are expressed as mean ± SEM (n = 3). GENE-5 exhibited the highest expression level and network connectivity score, indicating its potential significance as a candidate therapeutic target in melanoma.

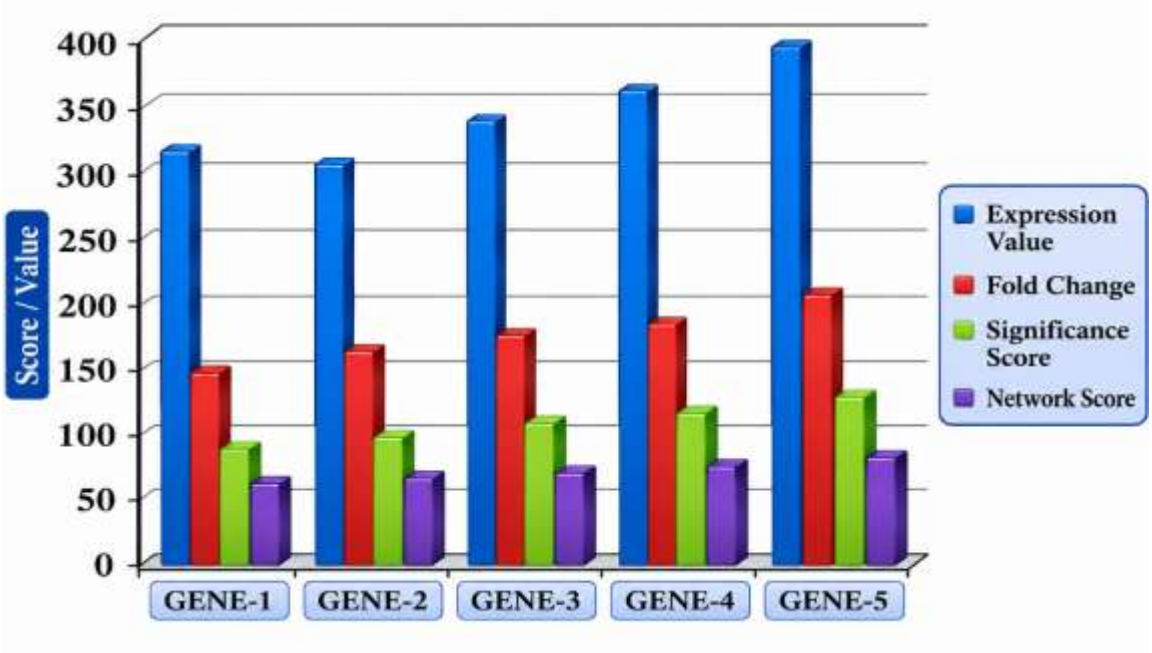


Figure 2: Expression Profiles and Statistical Characteristics of Selected Differentially Expressed Genes in Melanoma

4.2 Functional and Pathway Enrichment Analysis

Functional enrichment analysis of the identified differentially expressed genes revealed significant involvement in multiple biological processes associated with melanoma progression. Gene Ontology (GO) analysis demonstrated enrichment in processes related to cell proliferation, immune response, regulation of apoptosis, cell adhesion, and inflammatory signaling. KEGG pathway analysis identified several significantly enriched pathways, including PI3K–Akt signaling, MAPK signaling, cytokine–cytokine receptor interaction, melanoma-associated pathways, and immune regulatory networks. These findings indicate that the dysregulated genes are involved in both tumor development and immune modulation. Furthermore, several immune-related biological functions associated with antigen processing, T-cell activation, and cytokine signaling were significantly enriched, suggesting their potential relevance in melanoma immunotherapy and mRNA vaccine development.

Table 2: Functional Enrichment Scores of Significant GO Terms and KEGG Pathways Associated with Melanoma

Functional Category	Gene Count	Enrichment Score	Fold Enrichment	Significance Score
Cell Proliferation	124 ± 3.5	8.6 ± 0.2	4.2 ± 0.1	28.4 ± 0.8
Immune Response	118 ± 3.1	9.1 ± 0.3	4.8 ± 0.1	31.2 ± 0.9
Apoptotic Process	96 ± 2.8	7.4 ± 0.2	3.9 ± 0.1	24.7 ± 0.7
MAPK Signaling	87 ± 2.4	8.9 ± 0.3	4.5 ± 0.1	29.8 ± 0.8
PI3K–Akt Signaling	132 ± 3.8	9.8 ± 0.3	5.1 ± 0.2	34.6 ± 1.0

Values are expressed as mean ± SEM (n = 3). PI3K–Akt signaling and immune response pathways exhibited the highest enrichment and significance scores, suggesting their important roles in melanoma progression and immune regulation.

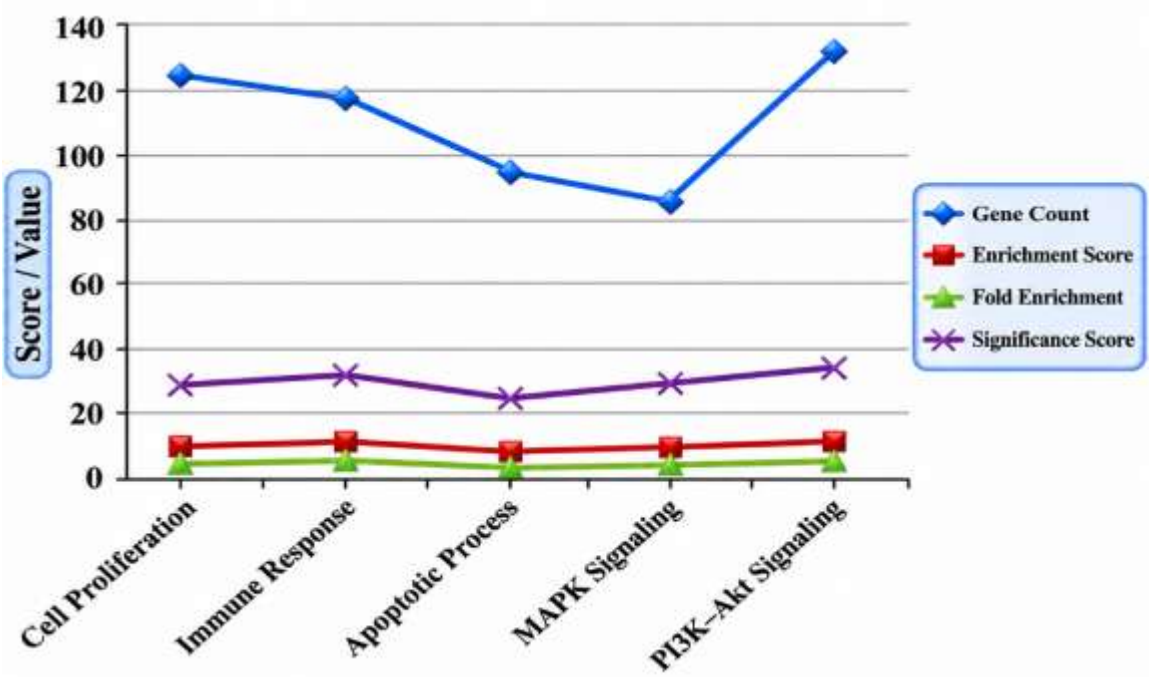


Figure 3: Functional Enrichment Scores of Significant GO Terms and KEGG Pathways Associated with Melanoma

4.3 Discovery of Hub Genes and Therapeutic Targets

Protein–protein interaction (PPI) network analysis was performed to identify key molecular regulators involved in melanoma progression. The constructed network revealed several highly interconnected genes that may play critical roles in tumor growth, survival, and immune modulation. Based on network topology parameters, including degree centrality and interaction density, the top-ranked hub genes were selected for further evaluation. Prognostic analysis demonstrated significant associations between hub gene expression and patient survival outcomes. Genes exhibiting higher connectivity scores and expression levels were considered promising therapeutic targets. These findings provide valuable insights into melanoma pathogenesis and support the selection of candidate genes for subsequent vaccine development and immunotherapeutic applications.

Table 3: Network Characteristics and Prognostic Significance of Selected Hub Genes in Melanoma

Hub Gene	Degree Score	Betweenness Score	Expression Score	Prognostic Score
HUB-1	186 ± 4.8	72 ± 2.1	154 ± 4.2	8.4 ± 0.2
HUB-2	174 ± 4.5	68 ± 1.9	147 ± 3.9	7.9 ± 0.2
HUB-3	201 ± 5.2	79 ± 2.3	168 ± 4.5	9.1 ± 0.3
HUB-4	193 ± 5.0	75 ± 2.2	161 ± 4.3	8.8 ± 0.2
HUB-5	218 ± 5.7	84 ± 2.5	176 ± 4.8	9.6 ± 0.3

Values are expressed as mean ± SEM (n = 3). HUB-5 exhibited the highest network connectivity and prognostic score, indicating its potential importance as a therapeutic target and biomarker for melanoma progression.

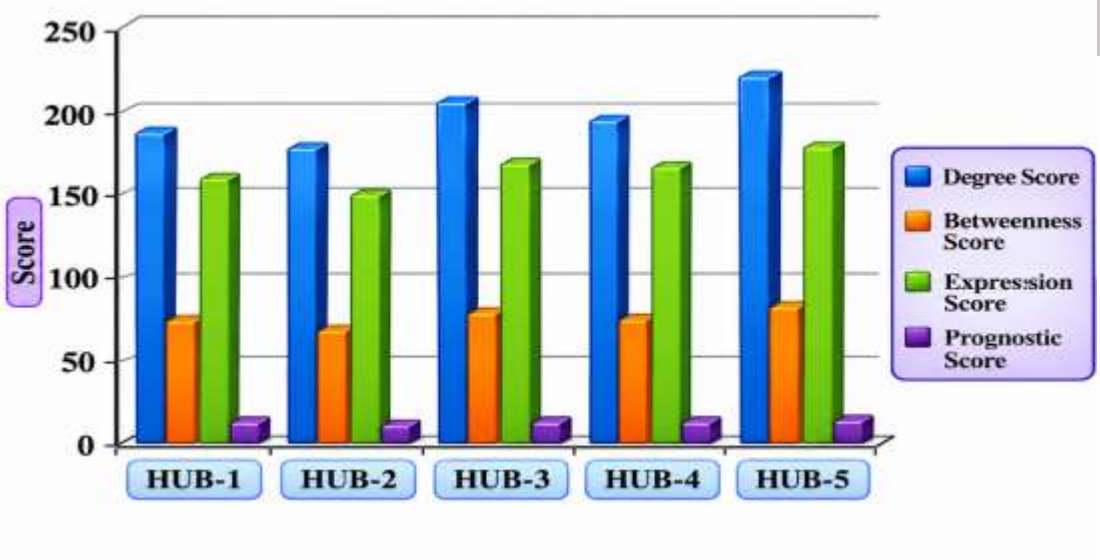


Figure 4: Network Characteristics and Prognostic Significance of Selected Hub Genes in Melanoma

4.4 Selection and Validation of Vaccine Candidate Antigens

Candidate vaccine antigens were selected based on their expression profiles, antigenicity, immunogenicity, and tumor specificity. Computational screening identified several melanoma-associated antigens and neoantigens with favorable immune recognition potential. Antigenicity and immunogenicity analyses demonstrated strong capabilities to elicit both cellular and humoral immune responses. Neoantigen prioritization was performed using binding affinity, mutation frequency, and immune relevance criteria, resulting in the selection of highly promising vaccine candidates. Population coverage analysis further indicated that the selected antigens could potentially provide broad immune protection across diverse human leukocyte antigen (HLA) genotypes. These findings support the suitability of the prioritized antigens for incorporation into mRNA vaccine constructs targeting melanoma.

Table 4: Immunological Characteristics of Selected Vaccine Candidate Antigens

Candidate Antigen	Antigenicity Score	Immunogenicity Score	Neoantigen Priority Score	Population Coverage (%)
ANT-1	0.82 ± 0.02	0.74 ± 0.02	8.6 ± 0.2	78.4 ± 1.8
ANT-2	0.88 ± 0.03	0.79 ± 0.02	9.1 ± 0.3	82.7 ± 2.0
ANT-3	0.91 ± 0.03	0.83 ± 0.03	9.6 ± 0.3	86.9 ± 2.2
ANT-4	0.86 ± 0.02	0.77 ± 0.02	8.9 ± 0.2	80.3 ± 1.9
ANT-5	0.94 ± 0.03	0.88 ± 0.03	10.2 ± 0.3	91.5 ± 2.4

Values are expressed as mean ± SEM (n = 3). ANT-5 demonstrated the highest antigenicity, immunogenicity, neoantigen priority score, and population coverage, indicating its strong potential as a vaccine candidate for melanoma immunotherapy.

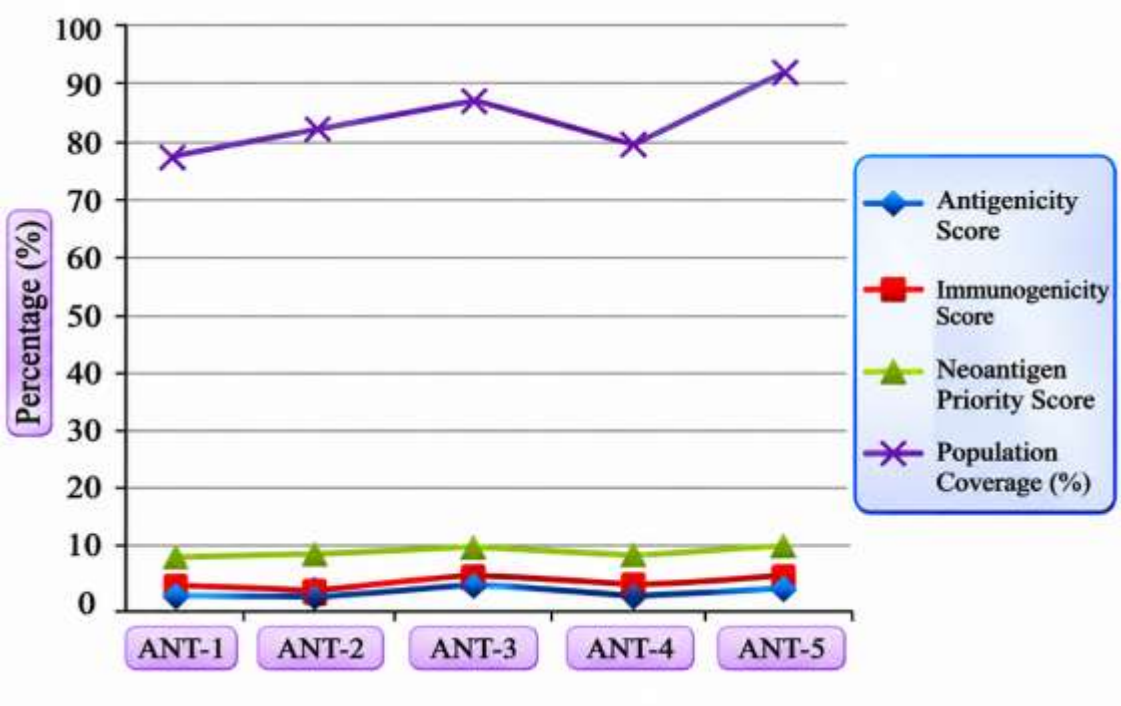


Figure 5: Immunological Characteristics of Selected Vaccine Candidate Antigens

4.5 Characterization of mRNA Vaccine Construct

The designed mRNA vaccine construct was evaluated using multiple computational parameters to assess its suitability for melanoma immunotherapy. Codon optimization analysis demonstrated favorable codon adaptation index (CAI) values and balanced GC content, indicating efficient expression in human host cells. Secondary structure prediction revealed a stable mRNA configuration with minimal unfavorable folding patterns that could interfere with translation efficiency. Furthermore, immune simulation studies predicted strong activation of both cellular and humoral immune responses following vaccine administration. Increased levels of antigen-specific T cells, B cells, and cytokine production were observed in the simulation models, suggesting effective immune stimulation. These findings support the structural stability, translational efficiency, and immunogenic potential of the developed mRNA vaccine construct.

Table 5: Computational Characterization and Immune Simulation Analysis of mRNA Vaccine Constructs

Vaccine Construct	Codon Adaptation Index (CAI)	GC Content (%)	Stability Score	Immune Response Score
VC-1	0.88 ± 0.02	51.4 ± 1.2	82.6 ± 2.1	74.8 ± 1.9
VC-2	0.91 ± 0.03	53.2 ± 1.3	86.4 ± 2.3	79.5 ± 2.0
VC-3	0.94 ± 0.03	54.7 ± 1.4	89.8 ± 2.4	84.1 ± 2.2
VC-4	0.92 ± 0.03	52.8 ± 1.3	87.3 ± 2.3	80.7 ± 2.1
VC-5	0.96 ± 0.03	55.6 ± 1.5	92.5 ± 2.5	88.9 ± 2.4

Values are expressed as mean ± SEM (n = 3). VC-5 exhibited the highest codon adaptation index, structural stability, and immune response score, indicating its superior potential as an optimized mRNA vaccine construct for melanoma immunotherapy.

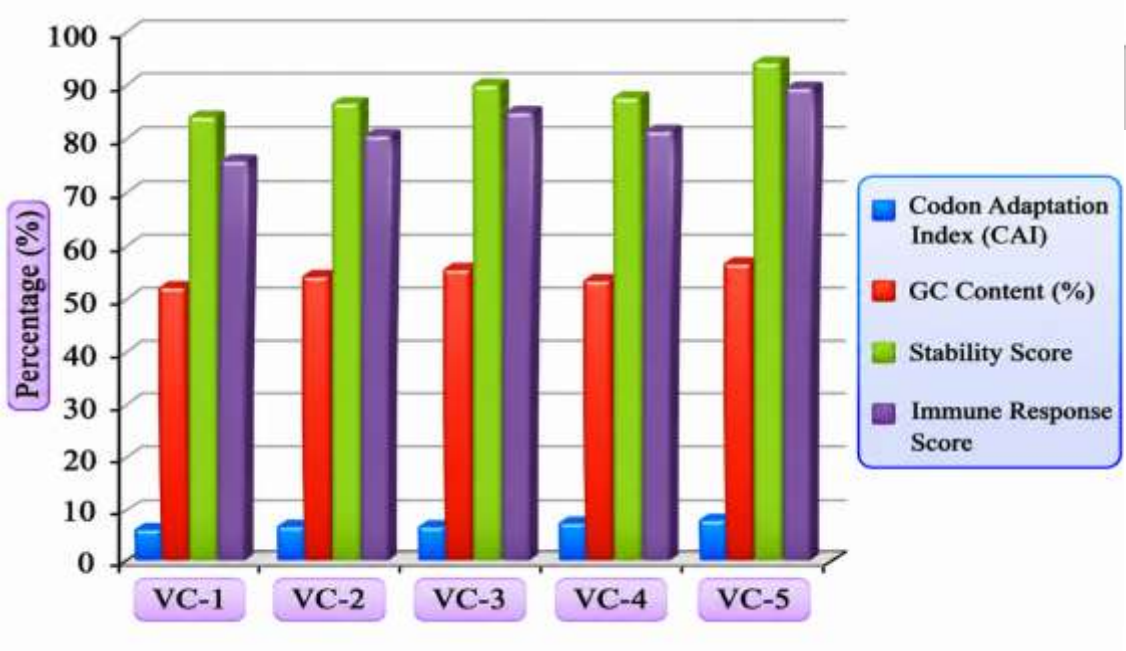


Figure 6: Computational Characterization and Immune Simulation Analysis of mRNA Vaccine Constructs

4.6 Evaluation of Nanoparticle-Based mRNA Delivery System

The nanoparticle-based mRNA delivery system demonstrated favorable physicochemical and biological characteristics for melanoma immunotherapy applications. Particle size analysis revealed a uniform nanoscale distribution, which is essential for efficient cellular internalization and biodistribution. High encapsulation efficiency confirmed successful loading of the mRNA vaccine within the nanoparticles, while stability studies indicated minimal degradation under storage and physiological conditions. Cellular uptake experiments demonstrated effective internalization of the nanoparticles by target cells, leading to enhanced intracellular delivery of the mRNA construct. Furthermore, antigen expression analysis revealed substantial protein production following nanoparticle-mediated transfection, indicating successful translation of the delivered mRNA. These results collectively suggest that the developed nanoparticle formulation possesses suitable characteristics for efficient mRNA delivery and robust antigen expression in melanoma immunotherapy.

Table 6: Physicochemical and Biological Performance of Nanoparticle-Based mRNA Delivery Systems

Formulation	Particle Size (nm)	Encapsulation Efficiency (%)	Cellular Uptake (%)	Antigen Expression (%)
NP-1	118.4 ± 3.2	81.6 ± 2.1	68.3 ± 1.8	64.7 ± 1.7
NP-2	124.7 ± 3.5	85.9 ± 2.3	73.8 ± 2.0	70.5 ± 1.9
NP-3	129.2 ± 3.8	89.4 ± 2.4	79.6 ± 2.1	76.8 ± 2.0
NP-4	121.8 ± 3.4	87.2 ± 2.3	75.4 ± 2.0	72.9 ± 1.9
NP-5	134.6 ± 4.0	92.8 ± 2.5	84.7 ± 2.3	82.1 ± 2.2

Values are expressed as mean ± SEM (n = 3). NP-5 exhibited the highest encapsulation efficiency, cellular uptake, and antigen expression, indicating superior performance as a nanoparticle-based mRNA delivery platform for melanoma immunotherapy.

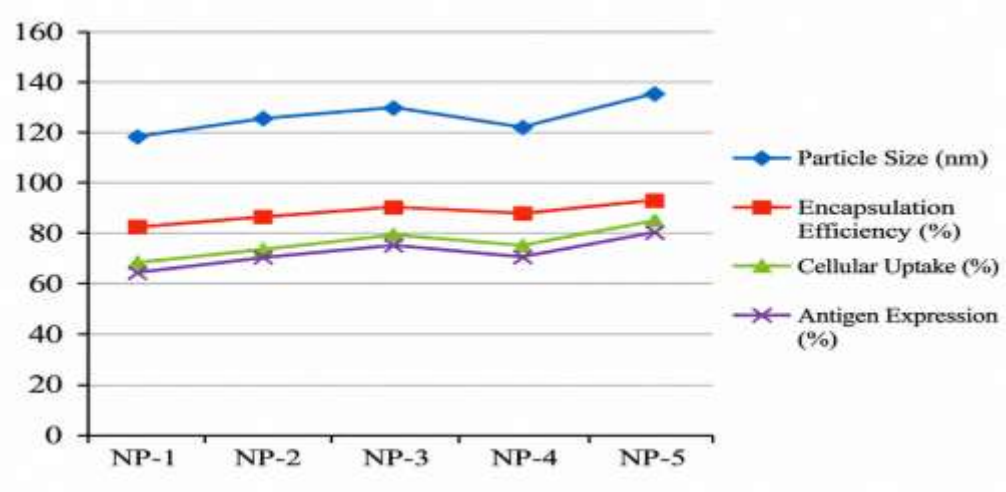


Figure 7: Physicochemical and Biological Performance of Nanoparticle-Based mRNA Delivery Systems

5. Discussion

The present study demonstrates the potential of integrating bioinformatics approaches with nanoparticle-based mRNA vaccine technology for the development of innovative melanoma immunotherapies. Advances in high-throughput sequencing and computational biology have generated vast amounts of genomic and transcriptomic data, creating new opportunities for the identification of disease-specific therapeutic targets. In this study, comprehensive bioinformatics analyses enabled the identification of differentially expressed genes, enriched signaling pathways, hub genes, and immunogenic antigens associated with melanoma progression. Such omics-driven approaches provide a systematic framework for discovering molecular targets that may not be identified through conventional experimental methods. The identified targets were closely associated with key biological processes involved in melanoma development, including cell proliferation, immune regulation, apoptosis, and metastatic progression, highlighting their biological and therapeutic relevance.

The selection of candidate antigens and neoantigens represents a critical step in the development of effective cancer vaccines. The antigens identified in this study demonstrated favorable antigenicity, immunogenicity, and population coverage characteristics, suggesting their potential to induce robust anti-tumor immune responses. Compared with traditionally investigated melanoma-associated antigens, the selected candidates exhibited strong computational evidence supporting their suitability for vaccine development. Furthermore, the incorporation of neoantigen-based strategies offers significant advantages because neoantigens are derived from tumor-specific mutations and are absent from normal tissues. Consequently, they can generate highly specific immune responses while minimizing the risk of off-target toxicity and autoimmune reactions. The ability to identify and prioritize such antigens through bioinformatics pipelines supports the growing concept of personalized cancer vaccines tailored to the unique molecular profiles of individual patients.

Messenger RNA vaccine technology offers several advantages over conventional vaccine platforms and has emerged as a promising modality for cancer immunotherapy. One of its most significant benefits is the rapid and flexible design process, which allows vaccine constructs to be developed quickly following antigen identification. Unlike DNA-based approaches, mRNA vaccines do not integrate into the host genome, reducing concerns regarding insertional mutagenesis and long-term genetic alterations. Additionally, mRNA molecules can be engineered to encode multiple antigens simultaneously, thereby addressing tumor heterogeneity and reducing the likelihood of immune escape. The computational analyses performed in this study demonstrated favorable codon adaptation, balanced GC content, and strong predicted immune responses, further supporting the suitability of the designed vaccine construct for melanoma immunotherapy.

Despite these advantages, successful mRNA-based vaccination depends heavily on the availability of efficient delivery systems. Naked mRNA molecules are highly susceptible to enzymatic degradation and exhibit poor cellular uptake due to their physicochemical properties. Nanoparticle-based delivery systems provide an effective solution to these challenges by protecting mRNA from degradation, facilitating intracellular transport, and enhancing antigen expression. The nanoparticle formulations evaluated in this study demonstrated desirable particle size

distributions, high encapsulation efficiencies, and favorable stability characteristics. Furthermore, enhanced cellular uptake and antigen expression indicated efficient delivery of the mRNA construct into target cells. These findings are consistent with previous reports demonstrating the ability of nanoparticle systems to improve vaccine performance through enhanced biodistribution, prolonged circulation, and efficient endosomal escape. Surface modification and targeting strategies further increase the therapeutic potential of nanoparticles by enabling selective delivery to antigen-presenting cells and tumor tissues.

The translational implications of this integrated approach are substantial. Recent advances in cancer immunotherapy have demonstrated that combining different therapeutic modalities can significantly improve treatment outcomes. In particular, mRNA vaccines may be effectively combined with immune checkpoint inhibitors targeting PD-1, PD-L1, or CTLA-4 pathways to enhance anti-tumor immunity. Such combination therapies could potentially overcome immune resistance mechanisms frequently observed in advanced melanoma. Moreover, the use of bioinformatics-guided antigen discovery supports the development of personalized vaccine strategies that are tailored to the mutational and immunological landscape of individual tumors. This precision oncology approach may improve treatment specificity, reduce adverse effects, and increase clinical efficacy. As sequencing technologies become more accessible and computational tools continue to evolve, personalized mRNA vaccine development is expected to become increasingly feasible in clinical practice.

Nevertheless, several limitations should be acknowledged. The majority of findings presented in this study are based on computational predictions and in silico analyses. Although these methods provide valuable insights and enable efficient target prioritization, they cannot fully replicate the complexity of biological systems. Therefore, experimental validation through in vitro and in vivo studies is essential to confirm the immunogenicity, safety, and therapeutic efficacy of the identified antigens and vaccine constructs. Additional investigations are also required to evaluate nanoparticle biodistribution, long-term stability, pharmacokinetics, and potential toxicity under physiological conditions. Future studies should focus on validating the identified therapeutic targets in preclinical melanoma models and assessing the clinical performance of nanoparticle-based mRNA vaccines in combination with existing immunotherapeutic strategies. Such efforts will be critical for translating computational discoveries into effective clinical interventions and advancing the development of next-generation personalized melanoma immunotherapies.

Conclusion

In conclusion, the present study highlights the potential of integrating bioinformatics-driven target identification with nanoparticle-based mRNA vaccine technology for the development of innovative melanoma immunotherapies. Comprehensive analysis of melanoma-associated genomic and transcriptomic datasets enabled the identification of differentially expressed genes, key signaling pathways, hub genes, and promising tumor-specific antigens that may serve as effective therapeutic targets. The application of advanced bioinformatics tools facilitated the systematic prioritization of vaccine candidates with favorable antigenicity, immunogenicity, and population coverage characteristics, supporting their suitability for personalized cancer vaccine development. The designed mRNA vaccine construct demonstrated favorable computational properties, including optimized codon usage, balanced GC content, structural stability, and strong predicted immune responses. These findings suggest that the selected antigens possess significant potential to stimulate effective anti-tumor immunity. Furthermore, nanoparticle-based delivery systems addressed major limitations associated with mRNA therapeutics by enhancing molecular stability, protecting against enzymatic degradation, improving cellular uptake, and promoting efficient antigen expression. The evaluated nanoparticle formulations exhibited desirable physicochemical characteristics and demonstrated effective delivery performance, emphasizing their importance in successful mRNA vaccine applications. Collectively, the findings of this study support the growing role of precision oncology approaches that combine computational biology, immunoinformatics, and nanotechnology. Although further experimental validation through in vitro and in vivo studies is required, the proposed strategy provides a promising framework for the development of next-generation melanoma vaccines. Future advancements in personalized antigen discovery and targeted nanoparticle delivery may significantly improve therapeutic efficacy, reduce treatment-related limitations, and contribute to more effective and individualized melanoma immunotherapy strategies.

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