Mathematical Biology and Bioinformatics
2024. V. 19. № 1. P. 155-168. doi: 10.17537/2024.19.155

========== **BIOINFORMATICS** ==========

A Bioinformatics Analysis for Unveiling Novel Long Non-Coding RNAs and their Regulatory Impact on Key Genes Associated with Vitiligo

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Abstract. Vitiligo involves the gradual disappearance of melanocytes, causing skin depigmentation. Long noncoding RNAs (lncRNAs), a type of noncoding RNA, are important for regulating inflammation and immunity. Despite this significance, there needs to be more published research on how lncRNAs are expressed in vitiligo cases and their potential roles in the biology of this skin condition. This study aims to elucidate the molecular landscape of vitiligo by analyzing gene expression profiles of vitiligo skin and normal skin. Two datasets, RNA-seq and microarray, were thoroughly investigated to identify differentially expressed (DE) genes and lncRNAs associated with vitiligo development. Functional enrichment analysis revealed biological processes and pathways influenced by dysregulated genes, highlighting intricate processes such as melanin biosynthesis and melanogenesis, shedding light on the complex regulatory networks involved in pigmentation and immune responses. Protein-protein interaction analysis highlighted significantly downregulated hub genes, including TYRP1, MLANA, MC1R, SLC45A2, PAX3, TYR, DCT, OCA2, PMEL, and SOX10, revealing significant functional relationships among identified hub genes within the network. RNA-seq data analysis uncovered DE-lncRNAs, emphasizing the regulatory role of lncRNAs in vitiligo. Moreover, the correlation analysis between the expression of lncRNAs and key genes associated with melanogenesis (OCA2, TYRP1, and PMEL) unveiled novel upregulated lncRNAs such as CRTC3-AS1, LCMT1-AS1, LINC02178 contributing to vitiligo development. Additionally, lncRNA-gene networks constructed based on key melanocyte-related genes provided insights into the molecular relationships relevant to vitiligo. Overall, this study offers a comprehensive understanding of vitiligo pathogenesis, identifying potential therapeutic targets and laying the foundation for future research in this critical area.

Key word: *vitiligo, sirtuin 1 gene, DE-lncRNAs, DEGs, DNA-seq and microarray data.*

INTRODUCTION

Vitiligo is an autoimmune disease characterized by a progressive loss of pigment in the skin and hair that has no known etiology [1]. A microscopic inspection reveals that the pigment-producing cells, known as melanocytes, are completely absent in this condition [2]. Many theories have been proposed to explain the etiopathogenesis of vitiligo, including oxidative stress, viral connections, biochemical mechanisms, genetic and immunological impacts, and neurological triggers [3].

Worldwide prevalence of vitiligo varies, with higher rates found in Oceania (1.2 %), Africa (0.4 %), and Europe (0.4 %) than in North America (0.2 %) and Asia (0.1 %) [4]. With no notable variations according to gender or ethnicity, the prevalence in Brazil ranges from 0.46 % to 0.68 % for the entire population. It usually manifests itself in people between the

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ages of 20 and 30, but it can strike people of any age, even the elderly and young. Up to 3.5 % of consultations in pediatric dermatology and 1.4 % to 1.9 % of consultations in dermatology are related to vitiligo, which is still a noteworthy concern [5–7].

Long non-coding RNAs (lncRNAs) are a group of RNA molecules found throughout mammals, and they typically consist of over 200 bases. LncRNAs do not function as templates for the synthesis of proteins, in contrast to RNAs that code for proteins [8]. Research examining the entire set of RNAs in mammals has revealed that lncRNAs greatly outnumber protein-coding RNAs in terms of diversity and quantity [9, 10]. However, the biological functions of only a small percentage of lncRNAs have been determined. A growing body of evidence suggests that lncRNAs play significant roles in a wide range of biological processes through intricate mechanisms such as gene regulation, owing to their ability to interact with DNA, RNA, or proteins [11–15]. For instance, these molecules can function as signaling entities, promoting transcription, or act as decoys to inhibit transcription. They also serve as epigenetic regulators and scaffolds, interacting with various proteins to form ribonucleoprotein complexes [16, 17].

At the transcriptional level, lncRNAs may directly engage in transcription by interacting with transcriptional complexes or DNA elements like promoters [18]. On a broader scale, they can modulate chromatin structures by recruiting enzymes that modify chromatin, thereby influencing the expression of a considerable number of genes. For example, lncRNAs can hinder the access of DNA-binding proteins to DNA recognition elements, leading to the induction or repression of transcription, depending on the nature of the targeted proteins. The regulation of gene expression by lncRNAs occurs at both transcriptional and posttranscriptional levels, showcasing their multifaceted roles in cellular processes [19].

The current understanding of how immune-related lncRNAs function suggests they play important roles in adjusting signals promoting inflammation and in immune cells' differentiation and polarization. These functions may be associated with the pathogenesis of vitiligo [20–22]. Hence, lncRNAs serve as crucial regulators in the initiation and progression of vitiligo, potentially offering new avenues for therapies or diagnostic approaches. In vitro, considerable research has been conducted on the regulatory roles of lncRNAs in melanocyte function. However, limited studies currently exist on the regulation of lncRNAs in vitiligo. Few published reports exist at this time about the expression profiles of lncRNAs in cases of vitiligo or their possible biological roles in this setting [23, 24].

This study delves into the intricate molecular landscape of vitiligo, an autoimmune condition marked by the progressive loss of skin and hair pigmentation. Many theories, such as oxidative stress, viral links, genetic effects, and immunological triggers, have been put out to explain the aetiology of vitiligo, but the cause is still unknown.In light of the substantial knowledge gaps, the emerging role of long non-coding RNAs (lncRNAs) in the pathophysiology of vitiligo is the main focus of this study. LncRNAs, a diverse group of RNA molecules, have been increasingly recognized for their regulatory roles in biological processes. While their functions in immune-related pathways hint at potential involvement in vitiligo, limited studies have explored their specific roles in this context. To address this gap, this study harnesses advanced genomics techniques, integrating RNA-seq and microarray datasets, to decipher the expression profiles of protein-coding genes and lncRNAs in vitiligo. By employing rigorous data analysis, functional enrichment studies, and protein-protein interaction analyses, this study aims to unravel the intricate network of genes, lncRNAs, and molecular pathways underlying vitiligo.

Furthermore, correlation analysis explores relationships between downregulated hub genes and differentially expressed lncRNAs in vitiligo. This comprehensive investigation not only enhances our understanding of the molecular basis of vitiligo but also sets the stage for identifying potential therapeutic targets and diagnostic markers in the pursuit of more effective treatment strategies.

METHODOLOGY

Overview of the study

This study aims to identify genes and long non-coding RNAs (lncRNAs) associated with vitiligo development, enhancing our understanding of potential therapeutic interventions. The study meticulously explores the molecular landscape of vitiligo, utilizing two relevant datasets from the NCBI Gene Expression Omnibus (GEO): RNA-seq PRJNA554241 and microarray GSE65127. The microarray dataset underwent rigorous analysis in R version 4.3.1, including PCA and differential expression analysis, leading to the identification of significant genes. Functional enrichment analysis revealed associated biological processes, molecular functions, and cellular components. Protein-protein interactions were explored through the STRING database, and hub genes were identified using the CytoHubba module in Cytoscape, uncovering the top 10 upregulated and downregulated hub genes. The RNA-seq data analysis focused on validating hub genes and long non-coding RNAs (lncRNAs) associated with vitiligo, ensuring robust data quality through preprocessing steps and differential expression analysis.

Furthermore, a correlation analysis between downregulated hub genes contributing to vitiligo and DE lncRNAs provided insights into intricate molecular relationships. Overall, this study integrates diverse genomic analyses to comprehensively characterize the molecular signatures of vitiligo, aiming to enhance our understanding and identify potential therapeutic targets for more effective treatment strategies.

Data collection

The selection of datasets for this study was based on their relevance to vitiligo. Two RNA-seq and microarray datasets were retrieved from the NCBI Gene Expression Omnibus (GEO) repository [25]. GEO serves as a comprehensive global repository covering diverse functional genomics datasets, encompassing various high-throughput data types, including micro-RNA profiles and next-generation sequencing data. The study incorporates the RNA-seq PRJNA554241 and the microarray GSE65127 dataset, generated using the Affymetrix Human Genome U133 Plus 2.0 Array platform. It comprises 10 patients (3 biopsies per patient: 1 lesional, 1 perilesional, and 1 non-lesional) and 10 healthy volunteers (1 biopsy in matched anatomical areas). Furthermore, the RNA-seq dataset with GEO ID PRJNA554241 consists of 10 vitiligo lesional vs. normal. This RNA-seq data was utilized to identify differentially expressed lncRNAs and validate the identified DEGs obtained from the microarray dataset.

Microarray dataset analysis

The microarray dataset was analyzed using the R version 4.3.1. The SDRF file, containing crucial sample information, was processed by assigning its row names to the corresponding column in the array data file, thereby creating an annotated data frame for subsequent analysis. Selected columns indicating sample phenotypes were extracted from the pData data frame. A log2 transformation was applied to the expression data, and the ratio of the first two Principal Components (PCs) was computed through Principal Component Analysis (PCA). Following summarization and background correction without normalization, row medians were calculated using the row Medians() function, and low-intensity transcripts were filtered based on a predefined threshold of 4. Genes with median intensities below this threshold were excluded, while those above were retained if they met the minimum sample requirement. Gene annotation was performed using the hgu133plus2.db package, eliminating probe IDs matching multiple genes. The limma package facilitated the application of empirical Bayes moderated *t*-statistics through the eBayes function, identifying differentially expressed genes (DEGs). A subset function was then used to extract DEGs with a *p*-value less than 0.05 and

logFC values less than -0.5 or greater than 0.5, focusing on the most statistically significant and biologically relevant genes.

Functional Enrichment Analysis

The functional enrichment analysis of the dysregulated genes was performed using EnrichR 3.1 package to unravel the Gene Ontology (GO) terms [biological processes (BP), molecular functions (MF), cellular components (CC)] and the KEGG pathways associated with the dysregulated genes. The EnrichR 3.1 package is widely used in the statistical programming language R 4.3.1 and was designed to analyze dysregulated genes [26]. The GO terms of both upregulated and downregulated genes were elucidated separately. The plotEnrich function generated the bar plots of the GO terms and KEGG pathways. In contrast, the enrichment terms were arranged based on minimum p-values, resulting in a comprehensive representation of the most significant annotations associated with dysregulated genes.

Protein-protein interactions and identification of hub genes

The protein-protein interactions (PPI) analysis was performed using the STRING database [27], comprising PPI information from databases, literature, and experimental validation. The DEGs obtained from the microarray data analysis were utilized as input in the STRING database by setting the default parameters, and the PPI genes that were subsequently employed in Cytoscape, a popular software platform used for the visualization and analysis of biological networks, were extracted. Further, the CytoHubba module in Cytoscape was used to identify the PPI network's top 10 upregulated and downregulated hub genes. The degree of each gene’s centrality within the network was determined, depicting the connections (edge) of each gene with other genes within the network; based on the centrality of the genes, the top 10 upregulated and downregulated hub genes were identified.

RNA-seq data analysis for hub genes validation and long non-coding RNA identification

RNA-seq data was utilized to identify dysregulated lncRNAs in vitiligo patients and validate the identified hub genes from the microarray dataset. To enhance the accuracy of subsequent analyses, preprocessing of the raw reads obtained from the sequencer was performed using the FastQC tool for quality assessment. FastP was then employed to eliminate poor-quality reads and contaminants, improving overall data quality. After cleaning, reads were aligned to the reference human genome (GRCh38) using the HISAT2 aligner, and duplicate reads were removed with Sambamba's markdup module. Following this, the StringTie tool measured gene expression levels, and Ballgown was used for differential expression analysis between vitiligo lesional patients and healthy controls. Significant genes were identified based on FPKM-normalized expression values, considering log-fold change (logFC) values and p-values and the results were visualized using a volcano plot.

Correlation analysis of downregulated hub genes and DE-lncRNAs

To uncover relationships between downregulated hub genes and DE-lncRNA in vitiligo, R version 4.3.1 was employed. The central function used for this analysis is cor.test, a fundamental function in base R. This function is systematically applied within nested loops to calculate Pearson correlation coefficients and corresponding p-values for pairs of lncRNAs and genes. A filtering step is implemented to focus on statistically significant associations, retaining only those correlation results where the p-value is below 0.05. This significance threshold is commonly used in hypothesis testing to ensure that the identified correlations are unlikely to occur by random chance. The filtered results, representing significant correlations, are then organized and stored in a new dataframe, providing valuable insights into robust molecular relationships relevant to vitiligo.

RESULTS

Identification of differentially expressed genes via microarray data analysis

The Ballgown package was utilized to conduct a differential gene expression analysis on a dataset GSE65127, comprising samples from vitiligo patients. In the dataset, 190 genes exhibited upregulation with a fold change (FC) value < 0.5 and a *p*-value < 0.05, while 238 genes showed downregulation with a fold change (FC) value < 0.5 and a *p*-value < 0.05. Figure 1,A and Figure 1,B.

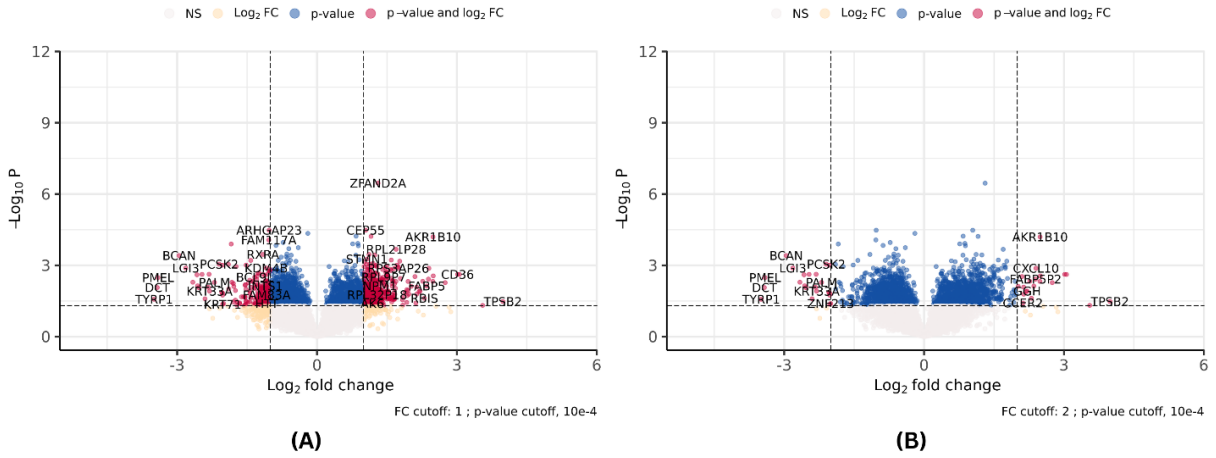

Fig. 1. Bioinformatics analysis images: **A**) Enhanced volcano plots representing differentially expressed genes in the vitiligo microarray dataset (GSE65127). Red colored dots represent upregulated and downregulated genes. Biologically significant genes are shown on the *x*-axis w.r.t Log2FC ± 0.5, and statistically significant genes are shown on the *y*-axis w.r.t Log10 *p*-value < 0.05. **B**) Enhanced volcano plots representing differentially expressed genes in the vitiligo RNA-seq dataset (PRJNA554241). Red colored dots represent upregulated and downregulated genes. Biologically significant genes are shown on the *x*-axis w.r.t Log2FC ± 1, and statistically significant genes are shown on the *y*-axis w.r.t Log10 *p*-value < 0.05.

Functional enrichment analysis of dysregulated genes identified through microarray dataset

The functional enrichment analysis of the dysregulated genes was performed in R programming language using the EnrichR 3.1 package to identify the GO terms, such as biological processes (BP), molecular functions (MF), cellular components (CC) and KEGG pathways that were significantly enriched among the dysregulated genes.

The GO enrichment analysis of the upregulated genes revealed the enriched BP such as Notch receptor processing; ligand-dependent, Notch receptor processing, amyloid precursor protein catabolic process, regulation of gene expression, amyloid precursor protein metabolic process, epidermal growth factor receptor signaling pathway, regulation of GTPase activity, membrane protein intracellular domain proteolysis, membrane protein ectodomain proteolysis, and positive regulation of hydrolase activity (see Supplementary Figure S1). Comparatively, the downregulated genes were involved in the dysregulation of the BP, such as regulation of circadian sleep/wake cycle, melanin biosynthetic process, proline transport, phenol-containing compound biosynthetic process, regulation of melanin biosynthetic process, positive regulation of cell differentiation, glycosaminoglycan biosynthetic process, positive regulation of multicellular organismal process, positive regulation of heart rate, and regulation of actin filament polymerization (Figure S2). Moreover, the upregulated genes that dysregulated the molecular functions involve mRNA binding, PDZ domain binding, alpha-mannosidase activity, ATPase binding, mannosidase activity, phosphate ion binding,

ribosomal large subunit binding, dynactin binding, *P*-type ion transporter activity, and calcium ion transmembrane transporter activity (Figure S3). However, the downregulated genes disrupted molecular functions such as sodium ion binding, sulfur amino acid transmembrane transporter activity, calcium ion binding, protein heterodimerization activity, metal ion binding, phospholipase A1 activity, carbonate dehydratase activity, phospholipase activity, protein homodimerization activity, and sequence-specific double-stranded DNA binding (Figure S4). Furthermore, the upregulated genes that showed dysregulation were predominantly expressed in Golgi-associated vesicle, intracellular membrane-bounded organelle, nucleus, bleb, gamma-secretase complex, endosome membrane, early endosome, proton-transporting V-type ATPase complex, trans-Golgi network transport vesicle, and nuclear outer membrane (Figure S5). Nonetheless, the downregulated genes were localized in melanosome, pigment granule, melanosome membrane, chitosome, pigment granule membrane, integral component of plasma membrane, extracellular membrane-bounded organelle, extracellular vesicle, polymeric cytoskeletal fiber, and collagen-containing extracellular matrix (Figure S6).

The enrichment analysis also revealed the KEGG pathways in which the genes were dysregulated. The upregulated genes were involved in the dysregulation of pathways including breast cancer, non-small cell lung cancer, epithelial cell signalling in *Helicobacter pylori* infection, melanoma, glioma, chronic myeloid leukaemia, pathways in cancer, oxytocin signalling pathway, human cytomegalovirus infection, and neurotrophin signalling pathway Supplementary Figure S7. In contrast, the downregulated genes were involved in the dysregulation of the pathways such as the PPAR signalling pathway, melanogenesis, protein digestion, and absorption, regulation of lipolysis in adipocytes, circadian rhythm, cGMP-PKG signalling pathway, arrhythmogenic right ventricular cardiomyopathy (ARVC), tyrosine metabolism, ECM-receptor interaction, and calcium signalling pathway Figure 2,A.

Protein-protein interactions and hub genes identification

The PPI analysis of the dysregulated genes was performed using the STRING database. This resulted in the network of genes showing significant functional relationships and potential interactions among the dysregulated genes, as mentioned in Figures S8 and S9. The CytoHubba plug-in within Cytoscape was employed to identify hub genes within the network. The top 10 upregulated and downregulated hub genes are represented in Figure 2,B and in Figure S10. Tables 1 and 2 present the top 10 upregulated and downregulated genes and their corresponding scores.

Table 1. Top 10 upregulated hub genes and their scores obtained through CytoHubba in Cytoscape

Upregulated Hub Genes	Scores
TP53	25
EGFR	16
FOS	10
KIF14	9
ECT2	9
FUS	9
E2F7	8
HELLS	8
ANLN	7
SRSF1	7

Table 2. Top 10 downregulated hub genes and their scores obtained through CytoHubba in Cytoscape

Downregulated Hub Genes	Scores
TYR	20
SOX10	20
DCT	16
SLC45A2	16
TYRP1	16
PAX3	16
OCA2	14
MC1R	14
PMEL	14
MLANA	13

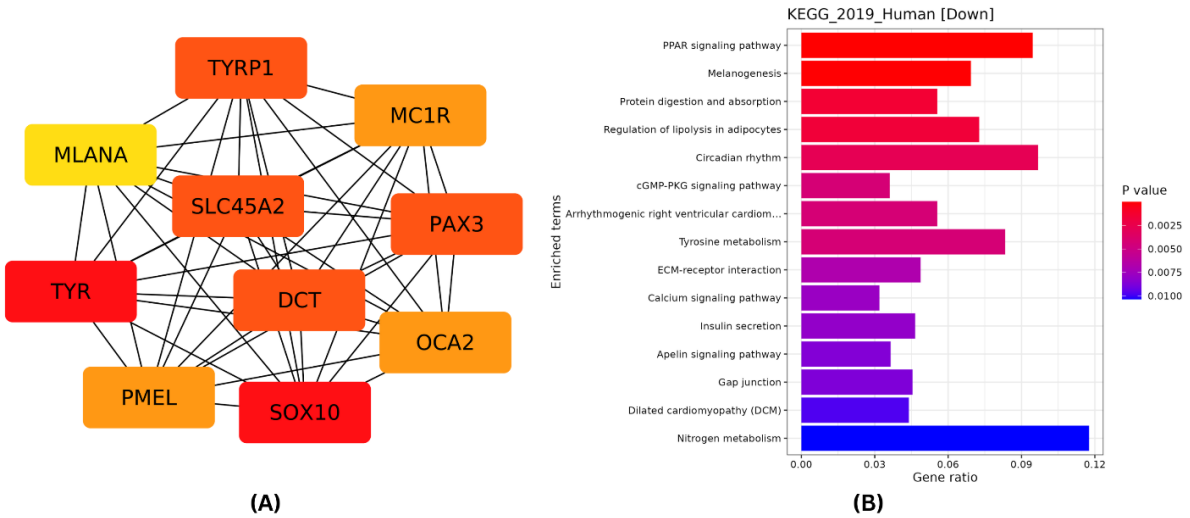

Fig. 2. Downregulated gene analysis: **A**) The bar chart represents KEGG pathways listed on the *y*-axis and the gene ratio displayed on the *x*-axis. Each bar corresponds to a specific KEGG pathway, and its length is proportional to the associated gene ratio, specifically representing the ratio of downregulated genes within each pathway. The gene ratio reflects the relative abundance of downregulated genes within each pathway among the differentially expressed genes. **B**) Visual representation of top 10 downregulated hub genes identified through CytoHubba plug-in within Cytoscape.

Identification of DEGs and DE-lncRNAs via RNA-seq data analysis

The Ballgown package was employed to perform a differential gene expression analysis on the PRJNA554241 dataset, which includes samples from individuals with vitiligo and adjacent normal skin. Within this dataset, 374 genes demonstrated upregulation, featuring a fold change (FC) value < 1 and a p-value < 0.05, while 201 genes displayed downregulation, characterized by a fold change (FC) value < 1 and a *p*-value < 0.05 Figure 1,B. Moreover, to validate the identified upregulated and downregulated hub genes via microarray, ANLN, and ECT2 were found in upregulated hub genes and TYRP1, MLANA, TYR, DCT, PMEL, and SOX10 as downregulated genes.

Among the significantly differentially expressed genes, 31 upregulated and 41 downregulated lncRNAs were identified. The top ten upregulated DE-lncRNAs were found to be CRTC3-AS1, LINC00664, UBE2Q2P2, LBX1-AS1, LINC02178, LINC02328, TNKS2-DT, LINC02073, COP1-DT, and PARD3-DT. Whereas the top 10 downregulated DE-

lncRNAs included CACNA1G-AS1, LINC00906, FOXD3-AS1, MIR2117HG, SLC25A30, CES1P1, LINC00663, SOX5-AS1, LINC00462, and DDX11L2. The top 10 upregulated and downregulated DE-lncRNAs with respect to logFC values are shown in Table 3 and Table 4, respectively.

Table 3. Top 10 upregulated DE-lncRNAs

Gene Names	*p*-value	logFC
CRTC3-AS1	0.010654505	5.325070836
LINC00664	0.038488054	4.472288172
UBE2Q2P2	0.006858498	3.330365887
LBX1-AS1	0.020599431	3.321082464
LINC02178	0.01231696	2.662123575
LINC02328	0.046358237	2.44643882
TNKS2-DT	0.018205728	2.312107544
LINC02073	0.008014591	2.261208477
COP1-DT	0.009261609	2.229306929
PARD3-DT	0.03928786	2.228286705

Table 4. Top 10 downregulated DE-lncRNAs

Gene Names	*p*-value	logFC
CACNA1G-AS1	0.012483524	-5.274495149
LINC00906	0.024140859	-4.249786336
FOXD3-AS1	0.003922218	-3.768659546
MIR2117HG	0.036869735	-3.666633391
SLC25A30	0.020617619	-3.443813028
CES1P1	0.004243899	-3.242904322
LINC00663	0.002214916	-3.222175616
SOX5-AS1	0.028758675	-2.987632247
LINC00462	0.002859643	-2.772964883
DDX11L2	0.036967011	-2.562111895

Correlation analysis of downregulated hub genes and DE-lncRNAs

To identify the DE-lncRNAs that specifically regulate the expression of downregulated hub genes related to melanogeneis correlation analysis was performed between the expression of lncRNAs and downregulated hub genes. Pearson correlation between expression of downregulated hub genes and DE-lncRNAs revealed four upregulated lncRNAs, including CRTC3-AS1, LCMT1-AS1, LINC02178, and LCMT1-AS1. Intriguingly, these lncRNAs were associated with the downregulation of OCA2, TYRP1, and PMEL genes.

Notably, the correlation values further highlighted the strength of these associations. CRTC3-AS1 and LCMT1-AS1 lncRNAs exhibited correlation values of -0.74 and -0.79, respectively, with OCA2 genes, accompanied by p-values of 0.03 and 0.01. Additionally, the correlation between LINC02178 lncRNA and the TYRP1 gene revealed a value of -0.77, with a p-value of 0.02. Similarly, LCMT1-AS1 demonstrated a correlation value of -0.75 with PMEL genes, supported by a p-value of 0.02, as mentioned in Table 5 and Figure 3,A.

Additionally, the exploration extended to identify more lncRNAs associated with the identified genes. Utilizing the NONCODE database, specific lncRNAs linked to key genes

were revealed. Noteworthy lncRNAs for the TYRP1 gene included lnc-TYRP1-5, lnc-TYRP1-4, and lnc-TYRP1-1. For the OCA2 gene, several lncRNAs were identified, including lnc-OCA2-5, lnc-OCA2-2, lnc-OCA2-1, lnc-OCA2-8, and lnc-OCA2-6. Similarly, for the PMEL gene, the identified lncRNAs were lnc-PMEL-1, lnc-PMEL-4, lnc-PMEL-3, and lnc-PMEL-2. Moreover, Cytoscape was used to construct lncRNA-gene networks based on the genes identified as closely related to melanocyte function, as mentioned in Figure 3,B.

Table 5. LncRNAs demonstrated correlation values with genes supported by *p*-value

lncRNA	Gene	Correlation	*p*_value
CRTC3-AS1	OCA2	-0.7496256047	0.0322390302
LCMT1-AS1	OCA2	-0.7977037209	0.01768370443
LINC02178	TYRP1	-0.7784829959	0.02285978845
LCMT1-AS1	PMEL	-0.7579848162	0.02931685583

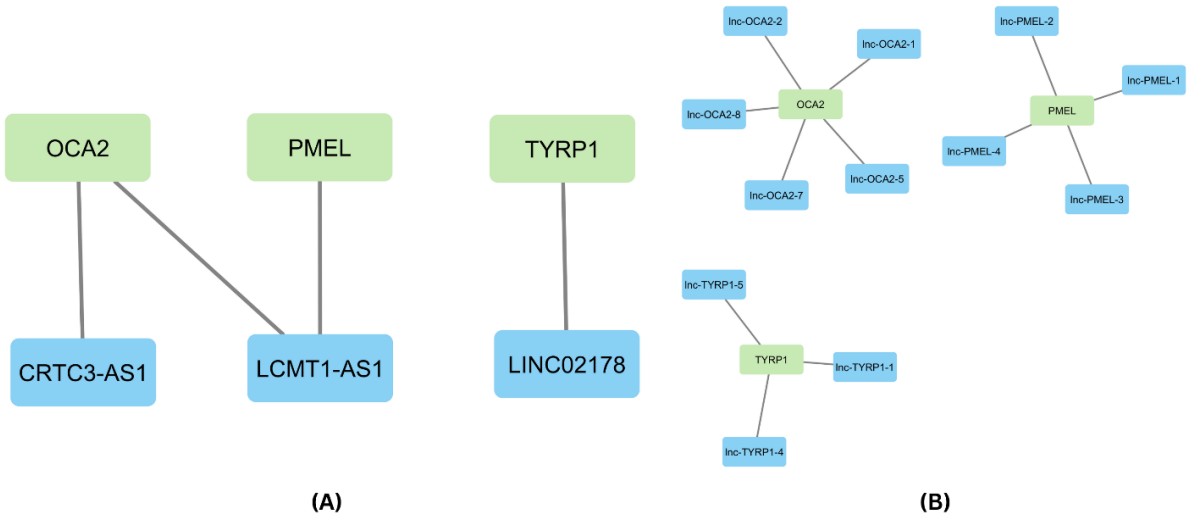

Fig. 3. Key genes analysis: **A)** The upregulated lncRNAs regulating the expression of key genes associated with vitiligo, identified through correlation analysis. Blue rectangle nodes represent lncRNAs, and green rectangle nodes represent genes. **B**) Other lncRNAs associated with key genes obtained through the NONCODE database.

DISCUSSION

Vitiligo, a prevalent skin disease resulting from depigmentation, is characterized by progressive dysfunction or reduced melanocyte numbers [28]. The loss of pigmentation in vitiligo is caused by autoimmune dysfunction, the removal of neurotoxic substances, and hereditary factors [29].

Long non-coding RNA (lncRNA), with a length exceeding 200 base pairs, plays a crucial role in modulating gene expression. Its mechanisms involve activating enhancer RNA, acting as a miRNA sponge, or binding to target genes to recruit chromatin-modifying enzymes [17]. For instance, Brahmbhatt et al. found that lncRNA MALAT1 suppresses miR-211, leading to the upregulation of sirtuin 1 expression and protecting keratinocytes from UVB-induced DNA damage [30]. While some studies have explored lncRNA's role in melanocytes, a comprehensive understanding of the expression profile and regulatory network of lncRNAs in vitiligo still needs to be developed [11, 31].

This study delved into the molecular landscape of vitiligo, utilizing RNA-seq and microarray datasets to comprehensively explore gene expression changes associated with this

skin disorder. Moreover, functional enrichment analysis uncovered the biological processes, molecular functions, and cellular components influenced by dysregulated genes. The upregulated genes exhibited enrichment in processes such as Notch receptor processing, amyloid precursor protein catabolic process, and regulation of gene expression, suggesting intricate molecular pathways at play. Conversely, downregulated genes were associated with circadian rhythm regulation, melanin biosynthesis, and positive cell differentiation regulation, highlighting the multifaceted nature of vitiligo pathogenesis.

Previous research has highlighted the importance of these processes in various cellular contexts. For instance, studies on the regulation of gene expression are a fundamental aspect of cellular function, and dysregulation in this process has been implicated in numerous diseases, including autoimmune conditions [32]. Moreover, circadian rhythm regulation, fundamental for cellular homeostasis, influences gene expression, metabolism, and stress responses. Previous research underscores its importance in promoting skin health, influencing melanocytes, and overall tissue functions [33]. Disruptions in circadian rhythms relate to various health issues, emphasizing its intricate interplay with cellular functionality. Integrating circadian biology insights enhances understanding of vitiligo pathogenesis [34]. Furthermore, melanin biosynthesis and positive cell differentiation regulation are crucial cellular processes. Studies, such as the work by Del Bino et al., have elucidated the molecular mechanisms governing pigmentation, emphasizing their significance in skin health [35].

Identification of dysregulated pathways provided additional insights into the molecular mechanisms underlying vitiligo. Downregulated genes were linked to pathways such as PPAR signaling and melanogenesis, shedding light on the complex regulatory networks involved in pigmentation and immune responses.

Furthermore, a previous study by Pei et al. provided evidence that the long non-coding RNA (lncRNA) UCA1 exerts a negative regulatory effect on melanogenesis in melanocytes. UCA1 was found to suppress MITF expression by negatively modulating the transcriptional activity of CREB, achieved through the inhibition of the cAMP/PKA, ERK, and JNK signaling pathways. This discovery underscores the potential of UCA1 as a therapeutic target for vitiligo [36]. In a separate study, Alhelf et al. observed a significant reduction in lncRNA TUG1 levels in vitiligo. They proposed that lncRNA TUG1 could impact melanogenesis by targeting miRNA-377, thereby influencing the expression of PPAR-γ and IL-17 in vitiligo [37]. The collective evidence from previous research and our study's findings suggests that lncRNAs may play a regulatory role in key melanogenesis genes within melanocytes, thereby contributing to the intricate development of vitiligo [23].

The comprehensive analysis of dysregulated genes in vitiligo sheds light on the intricate molecular landscape associated with this skin disorder. The PPI analysis conducted using the STRING database and CytoHubba plug-in within Cytoscape, revealed significant functional relationships and identified hub genes within the network. Notably, the top 10 upregulated hub genes included TP53, EGFR, FOS, KIF14, ECT2, FUS, E2F7, HELLS, ANLN, and SRSF1, while the downregulated hub genes comprised TYR, SOX10, DCT, SLC45A2, TYRP1, PAX3, OCA2, MC1R, PMEL, and MLANA.

Simultaneously, the study delved into the identification of DEGs and DE-lncRNAs through RNA-seq data analysis. The Ballgown package was employed to analyze the PRJNA554241 dataset and identified 374 upregulated and 201 downregulated genes.

The correlation analysis of downregulated hub genes and DE-lncRNAs has uncovered novel and intriguing associations that shed light on the intricate regulatory landscape of vitiligo. Remarkably, the upregulated lncRNAs, including CRTC3-AS1, LCMT1-AS1, LINC02178, and LCMT1-AS1, exhibit negative correlations with downregulated gene OCA2, TYRP1, and PMEL. The strength of these associations, elucidated by correlation values and p-values, introduces a novel dimension to our understanding of vitiligo pathogenesis.

Furthermore, lncRNA genes networks were constructed through Cytoscape based on the genes identified (TYRP1, OCA2, PMEL) that are closely related to the function of

melanocytes. In melanogenesis, TYRP1, regulated by the Microphthalmia-associated transcription factor (MITF), is a key protein for melanin production. MITF orchestrates the transcriptional events necessary for TYRP1, along with other pigmentation enzymes like tyrosinase (TYR) and dopachrome tautomerase (DCT). These enzymes, essential for melanin synthesis, possess specific binding sites susceptible to MITF's transcriptional control, highlighting MITF as a central regulator in TYRP1 transcription and melanin formation [38].

Previous studies reported that PMEL, also known as Pmel-17 or gp100, plays a crucial role in vertebrate pigmentation. Du et al. identified that the transcription of MLANA and PMEL is regulated by MITF in both melanocytes and melanoma [39, 40]. The OCA gene is crucial in oculocutaneous albinism (OCA), a rare disorder marked by reduced melanin biosynthesis. OCA2, associated with OCA, has been linked to an elevated risk of malignant melanoma, highlighting potential implications for skin cancer susceptibility [41].

Therefore, this research marks a novel exploration into the intricate world of vitiligo pathogenesis, focusing on the underexplored realm of lncRNAs and genes and their role in regulation. The limited prior work in this specific domain emphasizes our study's uniqueness and pioneering nature. By uncovering potential associations between hub genes and lncRNAs, this study introduces a novel perspective that may significantly contribute to the understanding of vitiligo. The identified gene-lncRNA connections represent a novel dimension in the field, paving the way for further investigations and underlining the need for more comprehensive research in this promising area.

CONCLUSIONS

In conclusion, this study provides a comprehensive exploration into the molecular landscape of vitiligo, uncovering key insights into the dysregulation of lncRNAs and the associated downregulation of crucial genes. Notably, the upregulation of specific lncRNAs, including CRTC3-AS1, LCMT1-AS1, and LINC02178, has been identified. These upregulated lncRNAs demonstrate significant negative correlations with downregulated genes such as OCA2, TYRP1, and PMEL. This downregulation implies disruptions in melanin synthesis, contributing to the depigmentation observed in vitiligo-affected skin. The strength of these associations, as evidenced by correlation values and *p*-values, introduces a novel dimension to our understanding of vitiligo pathogenesis, highlighting the potential regulatory role of lncRNAs in the observed gene expression changes. The identification of specific lncRNAs associated with key genes, along with the construction of lncRNA-gene networks, adds a layer of complexity to our understanding of the regulatory networks governing vitiligo. This study expands our knowledge of the molecular intricacies involved in vitiligo and sets the stage for future research to elucidate the functional roles of these lncRNAs and genes in the context of melanocyte biology and pigmentation. The findings presented here contribute to a more detailed understanding of vitiligo pathogenesis, opening avenues for targeted therapeutic interventions.

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Received 23.02.24.
Revised 13.04.2024.
Published 01.05.2024.