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ORIGINAL ARTICLE

Prediction of the engendering mechanism and specific genes of primary melanoma by bioinformatics analysis

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ABSTRACT

Objective: Our aim was to explore the engendering mechanism and gene targets for melanoma.
Methods: The microarray data of GSE46517 were downloaded from the Gene Expression Omnibus database. The differentially expressed genes (DEGs) between primary melanoma samples and normal controls were analyzed using the GEO2R online tool. The screened DEGs were mapped to a protein–protein interaction network based on the Search Tool for the Retrieval of Interacting Genes database. The functions and pathways involved with DEGs were analyzed using the Database for Annotation Visualization and Integrated Discovery software) online tools. Then, the DEGs were further annotated via the TRANSFAC, Tumor-Suppressor Gene, and Tumor-Associated Gene databases.
Results: A total of 1095 DEGs including 511 upregulated genes and 584 down-regulated ones were screened out. The nodes of *CCL5*, *ISG15*, *CDKN2A*, *EGFR*, and *ERBB2* showed a high connectivity degree in protein–protein interaction networks and were mainly enriched in Biological Process GO terms such as the regulation of catalytic activity and cell adhesion, as well as the pathways of cytochrome P450. The DEGs were classified into 31 transcription factors and 43 downregulated tumor associated genes.
Conclusion: Catalytic activity, cell adhesion, and the cytochrome P450 associated pathways are dysregulated in the melanoma formation. The significant nodes such as *ISG15*, *IRF4*, *ERBB2* and *EGFP* may be potential targets for primary melanoma treatment.

Introduction

Melanoma is a type of skin cancer characterized by the neoplasm development of pigment-producing cells called melanocytes. Melanoma is common in the skin, but may also develop in the eyes, ears, gastrointestinal tract, leptomeninges, oral and genital mucous membranes.[1] Melanoma is among the most rapidly increasing cancers of the white population in the USA.[2] It is estimated that 61,300 new cases are expected to be diagnosed in 2013.[3] Melanoma has metastatic potential and the median survival for metastatic melanoma patients is only 6–9 months.[4] Melanoma poses a burden to public health. A good understanding of genetic events underlying melanoma development can lead to the success of targeted therapies in melanoma treatment.[5] Melanoma pathogenesis has been proved to be driven by genetic mutation. Curtin et al[6] indicated that the mutations of *BRAF* or *NRAS* are common in the majority of melanomas without chronic sun-induced damage in skin. Reports showed that *MITF*, *CCND1*, *BRAF*, *CDKN2A*, and *PTEN* are some of the validated oncogenes and tumor suppressors selectively targeted by focal copy number-changing aberrations in melanoma.[7–9] Furthermore, microarray technology has been applied for investigating gene expression patterns for melanoma. Lawrence et al[10] developed microarray data of GSE46517 and proposed that chromosome 10, which encodes multiple tumor-suppressive functions, is frequently lost in human melanoma.[10] With the microarray data of GSE8041 and GSE46517, McCorkle et al[11] found that NME1 regulated the expression of genes that related with the metastasis and outcomes of patients with melanoma. However, the systematic studies of the aberrant gene expression of melanoma

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are rare and the molecular mechanism of melanoma development remains unclear.

Therefore, in the present study, we downloaded the publicly available microarray data (GSE46517) from the Gene Expression Omnibus (GEO) database and performed comprehensive analysis of gene expression patterns. The aim of this current work was to explore the potential molecular mechanism of melanoma and uncover the candidate gene targets for melanoma treatment.

Methods

Gene expression profiles

The publically available dataset (accession number: GSE46517) were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/).[12] The expression profiles data were developed from 121 samples, including 73 metastatic melanoma samples, 31 primary melanoma samples, one epithelial melanocyte sample, and seven normal skin samples. In the present work, the data from the 31 primary melanoma samples and seven normal skin samples were selected for further analysis based on the Affymetrix Human Genome U133A 2.0 Array (HG-U133A_2) platform.

Differentially expressed gene screening

The differentially expressed genes (DEGs) between primary melanoma and the normal controls were analyzed using the web tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html). Differences in gene expression values were evaluated by t test. Multiple testing correction was performed using the Benjamini–Hochberg method.[13] Finally, the DEGs with t ($\log_2$ fold change) >1 and adjusted $p < 0.05$ were screened.

Protein–protein interaction network construction

The Search Tool for the Retrieval of Interacting Genes (STRING) database is a collection of protein interaction data. It provides function and scores for protein pairs.[14] The protein interaction pairs of the screened DEGs were analyzed via the STRING database. Protein pairs with required confidence (combined score) >0.9 were screened for protein–protein interaction (PPI) network construction. The network was visualized by Cytoscape software.[15] The hub nodes in the PPI network were then identified based on the connectivity degree (number of neighbors) in the network statistics according to the scale-free attribute.[16]

Functions and pathways enrichment of DEGs

The Gene Ontology (GO) database is an open source for function annotation of a scale of genes.[17] The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database is a collection of various biochemical pathways.[18] The significantly overrepresented GO terms in biological process (BP) and pathways of DEGs were analyzed using the database for annotation, visualization and integrated discovery.[19] A p value < 0.05 was set as cut-off criterion.

Gene function annotation

The potential transfer factors (TFs) among DEGs were screened based on the TRANSFAC database.[20] The oncogenes and anti-oncogenes of the DEGs were determined based on the Tumor-Suppressor Gene (TSGene)[21] and Tumor-Associated Gene (TAG) databases according to the method described by Chen et al.[22]

Results

DEG screening

A total of 1095 genes corresponding to 1349 probes were identified to be differentially expressed, which included 511 upregulated DEGs and 584 downregulated genes. The detailed information is listed in Table 1.

PPI network

The PPI networks for upregulated and downregulated DEGs were constructed respectively. The PPI network for upregulated genes contained 146 nodes and 278 edges (Figure 1A). As shown in Figure 1B, there were 194 nodes and 265 edges in the PPI network for downregulated genes. After the connectivity degree analysis, the top 20 nodes with high degrees for the up- and downregulated PPI network were screened (Table 2). The connectivity degrees of the Top 20 nodes in the upregulated and downregulated PPI were all higher than 6.

Functions and pathways enrichment analysis of DEGs

To explore the functions of the DEGs, the 511 upregulated and 584 downregulated genes were subjected to GO-BP and KEGG pathway enrichment analysis. As shown in Table 3, the significantly enriched GO terms for upregulated genes were mainly related with the regulation of catalytic activity (GO:0050790), innate immune response (GO:0045087), chemotaxis (GO:0006935), and the regulation of immune response (GO:0050776). The downregulated genes were mainly enriched in cell adhesion (GO:0007155), response to wounding (GO:0009611), the positive regulation of gene expression (GO:0010628), and the enzyme linked receptor protein signaling pathway (GO:0007167). KEGG pathway analysis (Table 4) showed that upregulated genes were mainly enriched in the chemokine signaling pathway (04062), the pathways in cancer (05200), and the cytokine–cytokine receptor interaction (04060). The significantly enriched pathways of downregulated genes were metabolic pathways (01100) and focal adhesion (04510). Notably, pathways of drug metabolism–cytochrome P450 and metabolism of xenobiotics by cytochrome P450 were enriched by the downregulated DEGs.

Functional annotations of DEGs

In order to analyze the aberrant gene expression pattern for TFs and TAGs in primary melanoma, further functional analysis and annotation for DEGs were performed. As shown in Table 5, 12 upregulated DEGs (such as *MITF*, *CDK2*, *STAT1*, *IRF4*, and *IRF7*) were identified to be TFs and 19 downregulated DEGs that determined to be TFs included *EGR3*, *SMARCA2*, *TBX3*, *VDR*, and *RXRA*. Furthermore, based on the TAG database, 20 upregulated DEGs were identified to be well-documented TAGs, of which *CCND2*, *CEP55*, *FOSL1*, and *IRF4* were oncogenes, and *MMP11*, *CDKN2A*, *LPL*, *EIF2AK2*, *MAD1L1*,

Table 1 The numbers of the upregulated and downregulated probes and differentially expressed genes.

	Probe counts	Gene counts
Upregulated	607	511
Downregulated	742	584
Total	1349	1095

Probe counts = number of probes with query signal; Gene counts = number of differentially expressed genes.

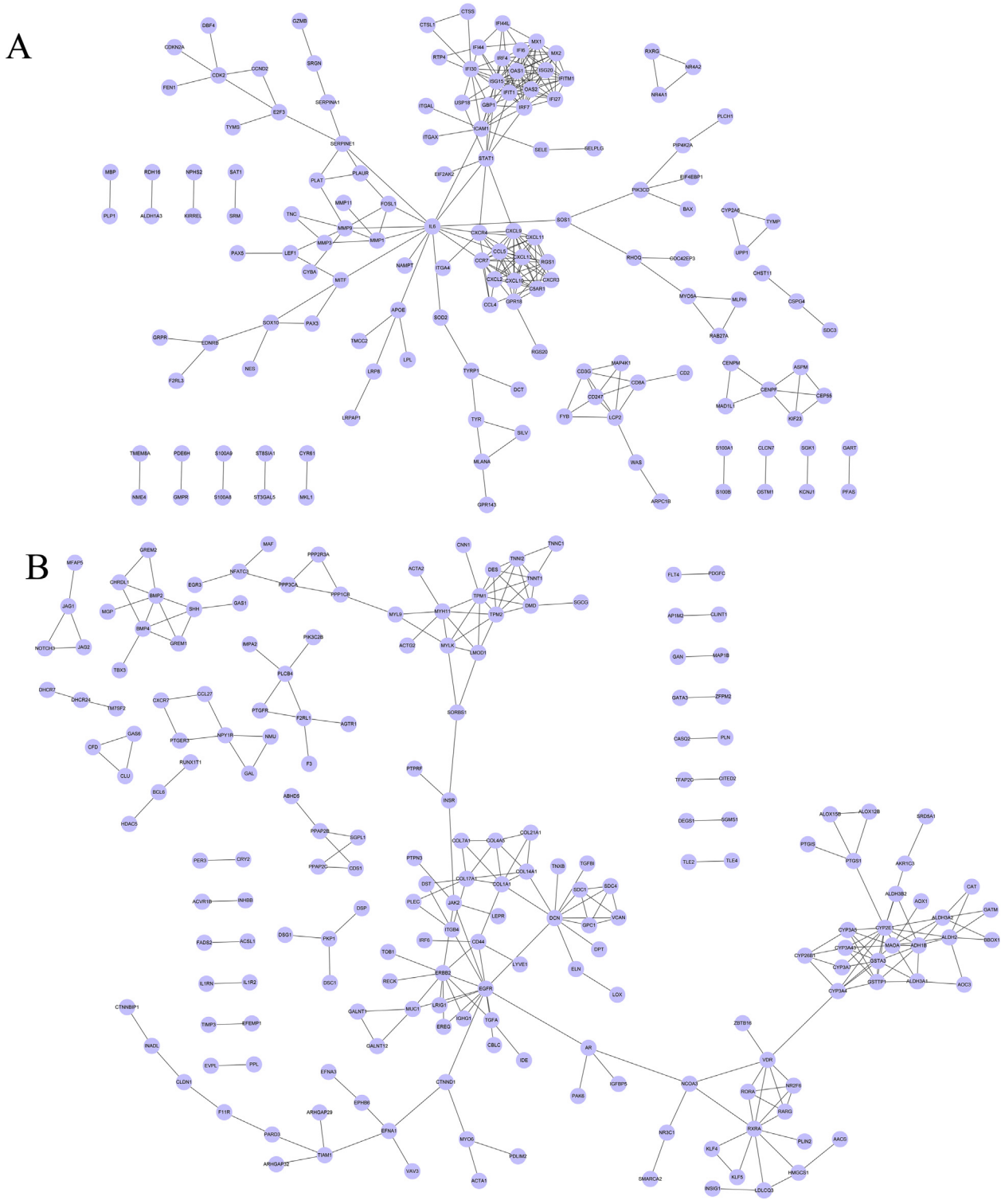

Figure 1 (A) Protein–protein interaction (PPI) network of the upregulated differentially expressed genes (DEGs). (B) PPI network of the downregulated DEGs. The interaction pairs of the screened DEGs were analyzed via the Search Tool for the Retrieval of Interacting Genes software. The PPI pairs were identified with the required confidence (combined score) >0.9 as a threshold, and the PPI network of these connections were constructed using Cytoscape software.

NR4A1, *SOD2*, *MAP4K1*, and *ISG15* were TSGenes. Meanwhile, 33 downregulated DEGs were identified to be TAGs, of which six genes (*VAV3*, *RUNX1T1*, *BCL6*, *NCOA3*, *ERBB2*, *EGFR)* were oncogenes and 18 genes (*PTPRF*, *TGFBI*, *SMARCA2*, *RECK*, *IGFBP5*, *GAS1*, *LOX*, *TIMP3*, *ARHGAP29*, *PTPN3*, *ZBTB16*, *CLU*, *KLF5*, *CTNND1*, *MUC1*, *EFNA1*, *BMP2*, *TPM1*) were TSGenes. However, the functions of the remaining nine DEGs (including the upregulated genes *BAX*, *PAX5*, *CSPG4*, *NR4A2*, *PAX3*, *CDK2,* and *OAS1* as well as the downregulated genes *MAF*, *MGP*, *TFAP2C*, *KLF4*, *ITGB4*, *SHH*, *TIAM1*, *CD44,* and *CYP3A4)* were not clear.

Table 2 Top 20 nodes with higher connectivity degrees in the protein–protein interaction network of the upregulated and downregulated differentially expressed genes.

Category	Gene	Degree	Gene	Degree	Gene	Degree	Gene	Degree
Up	ISG15	16	OAS1	13	IFI6	11	CXCL11	10
Up	IRF7	15	MX1	13	CCR7	11	IRF4	10
Up	IFIT1	15	CCL5	12	ISG20	11	CXCL10	10
Up	OAS2	14	CXCL9	12	MX2	11	ICAM1	10
Up	IL6	14	CXCL13	11	IFITM1	10	IFI27	10
Down	CYP2E1	13	TPM1	9	GSTTP1	7	COL17A1	7
Down	EGFR	13	ADH1B	8	ALDH2	7	BMP2	6
Down	DCN	11	GSTA3	8	COL1A1	7	DMD	6
Down	RXRA	10	TPM2	8	MAOA	7	TNNT1	6
Down	ERBB2	10	VDR	7	MYH11	7	ALDH3A2	6

Down = downregulated; Up = upregulated.

Table 3 Top 10 enriched gene oncology functions in biological processes of the differentially expressed genes.

Category	Term	Description	Count	*p*
Up	GO:0050790	Regulation of catalytic activity	37	3.33E-08
Up	GO:0045087	Innate immune response	32	2.73E-12
Up	GO:0006935	Chemotaxis	24	3.68E-09
Up	GO:0050776	Regulation of immune response	24	1.34E-07
Up	GO:0050900	Leukocyte migration	23	7.77E-16
Up	GO:0009615	Response to virus	23	1.33E-15
Up	GO:0008284	Positive regulation of cell proliferation	23	1.48E-07
Up	GO:0030334	Regulation of cell migration	18	8.77E-08
Up	GO:0042110	T cell activation	17	3.57E-08
Up	GO:0045944	Positive regulation of transcription from RNA polymerase II promoter	17	0.000645
Down	GO:0007155	Cell adhesion	41	6.65E-12
Down	GO:0009611	Response to wounding	39	7.47E-09
Down	GO:0010628	Positive regulation of gene expression	38	2.49E-08
Down	GO:0007167	Enzyme linked receptor protein signaling pathway	37	3.15E-10
Down	GO:0001568	Blood vessel development	30	2.58E-12
Down	GO:0030198	Extracellular matrix organization	28	4.44E-16
Down	GO:0071495	Cellular response to endogenous stimulus	26	8.29E-06
Down	GO:0030334	Regulation of cell migration	25	1.05E-10
Down	GO:0043588	Skin development	24	1.42E-12
Down	GO:0008285	Negative regulation of cell proliferation	24	1.23E-07

Down = downregulated; Up = upregulated.

Table 4 Top 10 enriched pathways of the differentially expressed genes.

Category	Term	Description	Count	*p*
Up	4062	Chemokine signaling pathway	14	1.26E-06
Up	5200	Pathways in cancer	13	0.001729
Up	4060	Cytokine-cytokine receptor interaction	11	0.002921
Up	4620	Toll-like receptor signaling pathway	9	2.90E-05
Up	4514	Cell adhesion molecules	8	0.001136
Up	5160	Hepatitis C	8	0.001193
Up	4810	Regulation of actin cytoskeleton	8	0.019073
Up	4670	Leukocyte transendothelial migration	7	0.00229
Up	4650	Natural killer cell mediated cytotoxicity	7	0.005551
Up	5323	Rheumatoid arthritis	6	0.003041
Down	1100	Metabolic pathways	35	0.010549
Down	4510	Focal adhesion	13	0.0003
Down	5200	Pathways in cancer	12	0.043972
Down	982	Drug metabolism - cytochrome P450	11	3.05E-07
Down	980	Metabolism of xenobiotics by cytochrome P450	10	2.06E-06
Down	4020	Calcium signaling pathway	10	0.004269
Down	4810	Regulation of actin cytoskeleton	9	0.037219
Down	5414	Dilated cardiomyopathy	8	0.000607
Down	4270	Vascular smooth muscle contraction	8	0.003141
Down	4520	Adherens junction	7	0.000852

Down = downregulated; Up = upregulated.

Discussion

To gain insight into the engendering mechanism of the primary melanoma, we systematically analyzed the gene expression profiles with bioinformatics analysis. A total of 1095 DEGs including 511 upregulated and 584 downregulated were screened in the current study. The biological functions of these DEGs were further explored based on GO function and pathway enrichment data. Further

Table 5 Functional classification on transcription factors (TFs) and tumor associated genes (TAGs) of the differentially expressed genes.

	TF counts	TF genes	TAG counts	TAG genes
Up	12	PAX5, RXRG, NR4A1, NR4A2, PAX3, LEF1, MITF, SOX10, CDK2, STAT1, IRF4, IRF7	20	CCND2, CEP55, FOSL1, IRF4, MMP11, CDKN2A, LPL, EIF2AK2, MAD1L1, NR4A1, SOD2, MAP4K1, ISG15, BAX, PAX5, CSPG4, NR4A2, PAX3, CDK2, OAS1
Down	19	EGR3, AR, RORA, SMARCA2, NFATC3, TBX3, MAF, GATA3, IRF6, KLF4, TFAP2C, HDAC5, KLF5, BCL6, NR3C1, NR2F6, VDR, RXRA, RARG	33	VAV3, RUNX1T1, BCL6, NCOA3, ERBB2, EGFR, PTPRF, TGFBI, SMARCA2, RECK, IGFBP5, GAS1, LOX, TIMP3, ARHGAP29, PTPN3, ZBTB16, CLU, KLF5, CTNND1, MUC1, EFNA1, BMP2, TPM1, MAF, MGP, TFAP2C, KLF4, ITGB4, SHH, TIAM1, CD44, CYP3A4

Down = downregulated; Up = upregulated.

analysis and annotation showed that 84 DEGs were well-documented TFs and TAGs, of which some were significantly involved in primary melanoma.

Our results showed that the GO term of regulation of catalytic activity (GO:0050790) was most significantly enriched by the upregulated genes. It is reported that tyrosinase is responsible for the melanin synthesis in skin, which results in the diversity of skin color.[23] The amount of melanin made and deposited in melanocytes is determined by the catalytic activity of tyrosinase instead of the abundance or tyrosinase gene activity. The tyrosinase, also known as melanocyte-specific enzyme, is involved in the rate-limiting step of the melanin synthesis pathway.[24,25] One sign of the melanoma in the early stage is the changes of shape and color of the existing moles, which is owing to the production of dark pigment and melanin by melanocytes.[26] In the current study, the regulation of catalytic activity was significantly enriched by the upregulated DEGs, suggesting that the catalytic activity was accelerated. This is in agreement with the previous reports.

It was also found that the drug metabolism—cytochrome P450 and metabolism of xenobiotics by cytochrome P450 pathways are significantly related to the downregulated DEGs in primary melanoma. The cytochromes P450 are known as key enzymes in cancer formation that mediate the metabolic activation of numerous precarcinogens.[27] Thus, these types of enzymes might play a similar role in the formation of melanoma. P450s are also regarded as an appropriate therapeutic target since they are expressed at higher levels in the tumor cells than in the surrounding normal tissue,[28] and they might be used in the treatment of primary melanoma.

Several DEGs with a high connectivity degree in the PPI network were identified in this study, such as *ISG15* (connectivity degree = 16), *IRF4* (degree = 10), *EGFR* (degree = 13), and *ERBB2* (degree = 10). *ISG15* and *IRF4* were found to be significantly upregulated and have been demonstrated to be TAGs. ISG15 encoded by the *ISG15* gene has a role in innate immunity and functions as a cytokine in lymphocytes and monocytes.[29] The cytokines ISG15 are expressed at high magnitude in melanomas and induce E-cadherin expression on dendritic cells (DCs).[30] E-cadherin expression on monocyte-derived DCs may potentially influence the migratory of DCs. DC infiltration is closely related with the induction of antitumor immunity that is associated with the prolonged survival and metastatic disease prevention.[31–33] GO enrichment analysis suggested that *ISG15* was mainly involved in the immune response and Type I interferon-mediated signaling pathway. Since Type I interferons were expressed at a high level in melanomas,[30] it is no wonder that many interferon-induced proteins were screened out as significantly upregulated DEGs including *IFI6*, *IFI27*, *IFIT1*, and *IFITM1*, as well as many interferon regulatory factors including *IRF4* and *IRF7*. Notably, *IRF4* was identified to be both a TF and a TAG upregulated in primary melanoma, which was consistent with the Duffy et al[34] finding that *IRF4* and nevus count represented a strong gene-by-age interaction with a strong tendency to melanoma. Thus, *ISG15* and *IRF4* play key roles in the progression of melanoma.

The gene *EGFR* encoding the epidermal growth-factor receptor tyrosine kinase was a significantly downregulated gene with the second highest connectivity degree in primary melanoma. Further annotation showed *EGFR* was an oncogene that was enriched in several significant BP-GO terms such as cell adhesion, enzyme-linked receptor protein signaling pathway, regulation of cell migration, and skin development. Members of the *EGFR* family have been reported to be overexpressed in skin cancer.[35] The EGFR phosphorylation induced by UV accelerated the development of skin cancer. Although *EGFR* is reported to be involved in progression and metastasis of a subset of melanomas,[36] it is still uncertain what role it plays in primary melanoma. In this paper, function and pathway enrichment analysis showed that *EGFR* participates in the cell adhesion process and the focal adhesion pathway. It was reported that cell adhesion molecules belonging to the integrin, cadherin, and immunoglobulin superfamilies are implicated in tumor progression of cutaneous melanoma.[37] Another report showed that during melanoma formation, there is a shift from Ecadherin expression to neural Ncadherin expression in melanocytes.[38] Hence, *EGFR* as a key component of signal transduction in focal adhesion may contribute to primary melanoma process by altering the expression of cadherin.

ERBB2, another significantly downregulated DEG was identified as an oncogene and showed close association with *EGFR* in the PPI network. *ERBB2,* also known as neu/HER-2, is another epidermal growth factor. The gene amplification and protein overexpression of *ERBB2* are associated with various human cancers such as medulloblastoma[39] and breast carcinomas.[40] Some evidence shows that the focal adhesion signaling pathway might participate in *ERBB2*-induced tumorigenesis.[41] Therefore, *ERBB2* interacting with *EGFR* may play a key role in focal adhesion signaling pathway in primary melanoma.

Although our studies predicted significant biological function, pathways and genes involved in melanoma formation, further experimental validation is warranted.

Conclusion

In summary, regulation of catalytic activity and cell adhesion, as well as the cytochrome P450 associated pathways may be crucial in melanoma formation. The significant nodes with high degrees such as *ISG15*, *IRF4*, *ERBB2*, and *EGFP* play critical roles in the progression of melanoma and give insight to the biomarker and target therapy for primary melanoma. However, numerous studies should be conducted to validate our findings.

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