Identification of key genes involved in the pathogenesis of cutaneous melanoma using bioinformatics analysis

Jianqin Chen[1],*, Wen Sun[2],*, Nian Mo[1], Xiangjun Chen[1], Lihong Yang[3], Shaozhong Tu[4], Siwen Zhang[1] and Jing Liu[3] (ID)

Abstract

Objective: Malignant melanoma is a highly invasive cancer whose pathogenesis remains unclear. We analyzed the microarray dataset GDS1375 in the Gene Expression Omnibus database to search for key genes involved in the occurrence and development of melanoma.

Methods: The dataset included 52 samples (7 normal skin and 45 melanoma samples). We identified differentially expressed genes (DEGs) between the two groups and used integrated discovery databases for Gene Ontology (GO) and Kyoto Gene and Genome Encyclopedia (KEGG) pathway analyses. In addition, we used the STRING and MCODE plugins of Cytoscape to visualize the protein-protein interactions (PPI) for these DEGs.

Results: A total of 509 upregulated and 618 downregulated DEGs were identified, which were enriched in GO terms including integrin binding, protein binding, and structural constituent of cytoskeleton, and in KEGG pathways such as melanogenesis, prostate cancer, focal adhesion, and renin secretion. Three major modules from the PPI networks and 10 hub genes were identified, including *CDC20*, *GNB2*, *PPP2R1A*, *AURKB*, *POLR2E*, and *AGTR1*. Overall survival was low when these six hub genes were highly expressed.

Conclusion: This bioinformatics analysis identified hub genes that may promote the development of melanoma and represent potential new biomarkers for diagnosis and treatment of melanoma.

[1]Guangzhou University of Chinese Medicine, Guangzhou, China
[2]Department of Dermatology, The First People's Hospital of Jingmen, Jingmen, China
[3]Department of Dermatology, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China

[4]Department of Dermatology, Beijing University of Chinese Medicine Shenzhen Hospital, Shenzhen, China

*These authors contributed equally to this work.

Corresponding author:
Jing Liu, Department of Dermatology, The First Affiliated Hospital of Guangzhou University of Chinese Medicine, 16 Airport Road, Guangzhou, Guangdong, 510000, China.
Email: ljgztcm@163.com

Background

Melanoma is a common skin tumor, and epidemiological studies have shown that the incidence of melanoma has increased dramatically in the past 50 years. In 2010, the incidence of melanoma in the United States was 35.4 and 24.2 cases per 100,000 men and women, respectively. In contrast, in the 1960s, the respective incidences were only 9.4 and 8.2 cases per 100,000.[1] The prognosis of advanced melanoma is poor and the median overall survival rate is only 23 months.[2]

Although great progress has been made in the diagnosis and treatment of melanoma (e.g., *BRAF* V600E inhibitors, early surgical resection), the long-term survival rate remains low in patients with advanced melanoma, mainly due to distant metastases.[3,4] Thus, there is an urgent need to elucidate the molecular mechanisms underlying the development and metastasis of melanoma to develop new and better therapeutic strategies.

Many recent studies have found that biomarkers can be used to screen for and diagnose skin melanoma. For examples, Song et al.[5] reported that CDKL1 (cyclin dependent kinase like 1) inhibits the growth and colony formation of melanoma cells by increasing apoptosis. Liu et al.[6] showed that the metastasis of melanoma can be inhibited by microRNA (miR)-425, which represses the PI3K-Akt pathway by targeting insulin-like growth factor-1. Kubic et al.[7] found that PAX3 (paired box 3) and FOXD3 (forkhead box D3) upregulate *CXCR4* (C-X-C motif chemokine receptor 4) expression in melanoma. However, the discovery of these biomarkers still fails to fully explain the mechanisms underlying the growth and metastasis of melanoma.

To better understand the molecular mechanisms of melanoma, we analyzed the microarray dataset GDS1375 from Gene Expression Omnibus (GEO), which contains expression data from both melanoma and normal tissues to identify differentially expressed genes (DEGs) and subsequently construct a protein–protein interaction (PPI) network to identify highly connected central genes for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. In addition, we performed an overall survival (OS) analysis to further elucidate the biological significance of the identified genes. The findings could provide new insights into molecular mechanisms related to melanoma pathogenesis and clues to develop better biomarker and therapeutic approaches for the cancer.

Materials and methods

Ethics and consent

This study used bioinformatics analysis and did not involve humans or animals. Therefore, local ethics committee approval and informed consent were not needed.

DEG identification

The microarray dataset GDS1375 was obtained from the Gene Expression Omnibus (GEO) database (https://www.

ncbi.nlm.nih.gov/geo/). It included 52 samples—7 normal skin samples and 45 melanoma samples. DEGs in the samples were identified via the limma package of R (www.r-project.org). The Bonferroni and Hochberg method was used to correct the *P*-values. Thresholds of $P < 0.02$ and $|\log_2$ (fold change)$| \geq 2$ were set. In addition, hierarchical clustering analysis of the DEGs was performed and volcano maps were plotted.

GO and KEGG pathway analysis

GO analysis is a common method for annotating genes and gene products as well as characterizing biological properties of high-throughput genome or transcriptome data. KEGG is a set of databases dealing with genomes, biological pathways, diseases, drugs, and chemicals.[8] DAVID (https://david.ncifcrf.gov/) is a web-based online bioinformatics tool designed to interpret the functions of a large number of genes or proteins.[9] We used these tools to analyze and visualize the core biological processes, molecular functions, cellular components, and pathways associated with these DEGs.

PPI network and module analysis

The search tool for retrieval of interacting genes (STRING; https://string-db.org/) is an online tool that assesses PPI.[10] To detect potential relationships between DEGs, we used the STRING plug-in in Cytoscape (https://cytoscape.org/) to map the interactive networks of the DEGs. The confidence score and the maximum number of interactors were set at ≥ 0.9 and 0, respectively. In addition, the MPI application was used to screen the PPI network in Cytoscape with a cutoff of 2, a node score cut-off of 0.2, a k-core of 2, and a depth of 100. Gene pathway analysis in each module was done using DAVID. In addition, 10 hub genes

were mapped using STRING with a confidence score of ≥ 0.4 and a maximum value of the interaction parameter of ≤ 5.

Survival analysis of hub genes and construction of miRNA-hub gene network

GEPIA (http://gepia.cancer-pku.cn/index.html) is a newly developed interactive web server for the analysis of gene expression from The Cancer Genome Atlas (TCGA) and Genotype Tissue Expression (GTEx) projects; we used GEPIA to analyze the survival response of the hub genes as boxplots. In addition, we identified the microRNA (miRNA) targets of the hub genes using Targetscan (http://www.targetscan.org/vert_72/). The most-matched miRNAs were used to construct a miRNA-hub gene network using Cytoscape.

Results

Identification of DEGs

Seven normal skin samples and 45 melanoma samples were analyzed in this study; 509 upregulated and 618 downregulated genes were identified, as well as 10 hub genes (Figure 1a and b).

GO function and KEGG pathway enrichment analysis

To gain a better understanding of the DEGs, we used DAVID for GO function and KEGG analysis. GO analysis showed that the DEGs were enriched in molecular function, integrin binding, protein binding, structural constituent of cytoskeleton, calcium ion binding, structural molecule activity, heparin binding, transcriptional activator activity, RNA polymerase II core promoter proximal region, actin binding, collagen binding, and cadherin binding involved in cell–cell adhesion (Figure 2a). In biological processes, enriched terms included
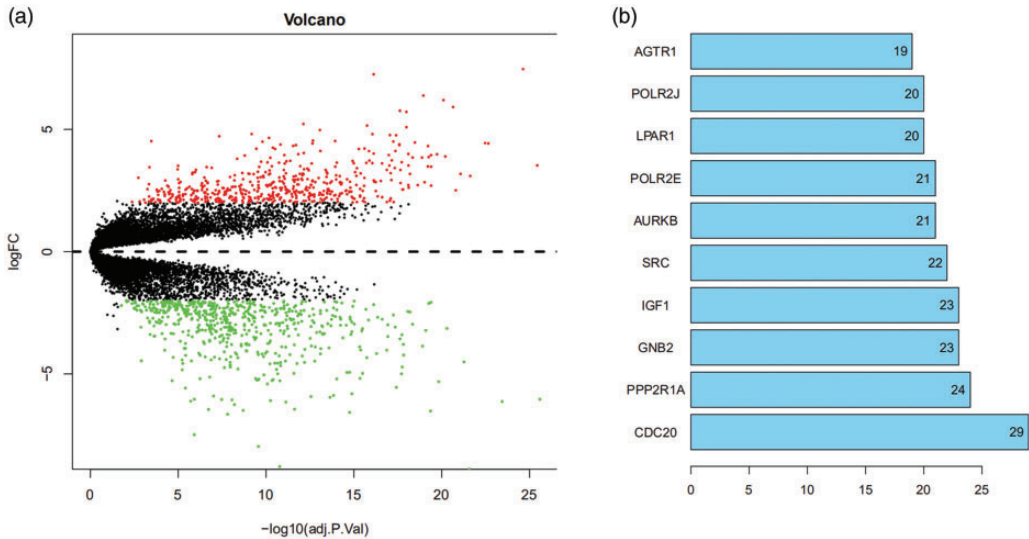
Figure 1. Differential expression profiles of mRNAs in cutaneous melanoma and control tissues. (a) The horizontal dotted line delimits up- and downregulation. Red and green dots represent significant mRNAs with fold change >2 and $P < 0.02$. Red dots represent up-regulated genes, green dots represent down-regulated genes. (b) Ten hub genes with their fold changes. FC, fold change.

epidermis development, cell adhesion, extracellular matrix (ECM) organization, ECM disassembly, keratinocyte differentiation, positive regulation of peptidyl-tyrosine phosphorylation, platelet degranulation, positive regulation of cell proliferation, positive regulation of protein kinase B signaling, and positive regulation of cellular protein metabolic process (Figure 2b). In addition, GO cell component analysis showed that the DEGs were significantly enriched in extracellular exosome, ECM, extracellular space, extracellular region, proteinaceous ECM, cell surface, cell–cell junction, melanosome, desmosome, and apical plasma membrane (Figure 2c). KEGG pathway analysis showed that the DEGs were enriched in melanogenesis, prostate cancer, focal adhesion, renin secretion, PI3K-Akt signaling pathway, arrhythmogenic right ventricular cardiomyopathy, ECM-receptor interaction, PPAR signaling pathway, and pathways in cancer and tyrosine metabolism (Figure 2d).

Hub genes and PPI network modules

Based on the STRING protein query information from the public database, we created PPI networks of the top 10 hub genes with high connectivity (Figure 3). In addition, we used the MCODE plug-in to select the first three modules. KEGG pathway enrichment analysis showed that the modules were primarily associated with cell cycle (Figure 3).

miRNA-hub gene network and prognostic value of hub genes

We screened the miRNA of the hub genes using Targetscan (http://www.targetscan.org/vert_72/). The most-matched miRNAs were used to make the miRNA-hub gene network (Figure 4). GEPIA (http://gepia.cancer-pku.cn/index.html) is a newly developed interactive web server for the analysis of RNA sequencing expression of 9736 tumors and 8587 normal samples from the TCGA and GTEx projects. Prognostic
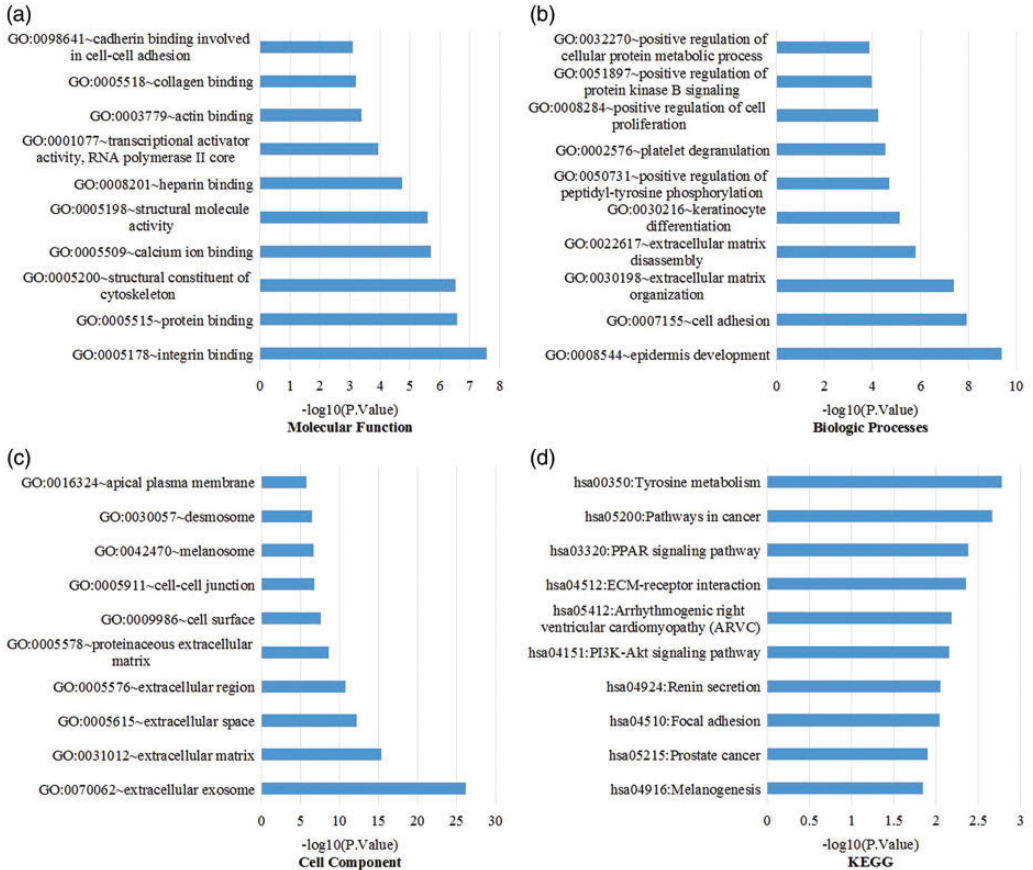
Figure 2. GO analysis and KEGG pathway analysis of differentially expressed genes. (a) Molecular functions, (b) biological processes, (c) cell components, and (d) KEGG pathways. GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

information associated with the 10 hub genes was available in GEPIA. We analyzed prognosis associated with the genes *CDC20*, *GNB2*, *PPP2R1A*, *AURKB*, *POLR2E*, and *AGTR1*; prognosis was poor when these genes were highly expressed (Figure 5).

Discussion

To illustrate the mechanisms underlying the pathogenesis of melanoma, we analyzed microarray expression profiles of melanoma tissues. Compared with normal skin samples, 1127 DEGs were identified in melanoma samples, including 509 upregulated and 618 downregulated genes.

The GO analysis revealed that DEGs are involved in melanogenesis, epidermis development, keratinocyte differentiation, extracellular matrix, melanosome, and integrin binding. The KEGG pathways showed that DEGs are mainly involved in melanogenesis, prostate cancer, and other pathways in cancer. In addition, we used the MCODE plug-in to select the first three modules. KEGG pathway analysis showed that the three most important modules were related to pathways in focal adhesion,
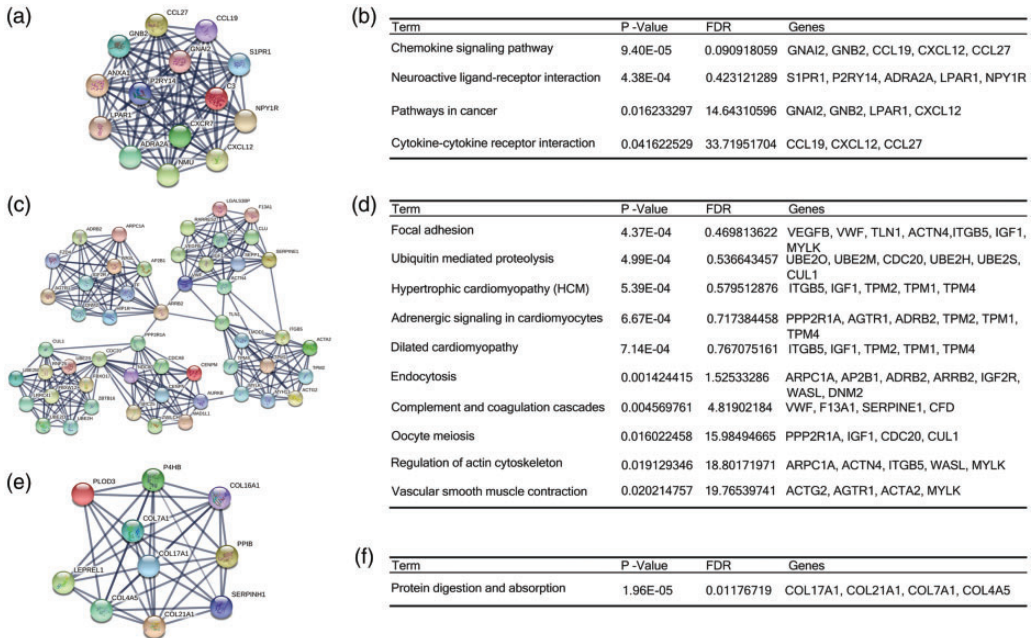
Figure 3. The protein–protein interaction networks of top three modules. Module 1 (a) and the enriched pathways of module 1 (b); module 2 (c) and the enriched pathways of module 2 (d); module 3 (e) and the enriched pathways of module 3 (f). FDR, false discovery rate.

cancer, and protein degradation and absorption.

To identify the hub genes, we selected 10 genes with the highest connectivity in DEGs in the PPI network. Survival analysis showed that 6 out of the 10 genes (*CDC20*, *GNB2*, *PPP2R1A*, *AURKB*, *POLR2E*, and *AGTR1*) were associated with prognosis. It is likely, therefore, that these genes are involved in the pathogenesis of melanoma and would be candidates for further functional analysis.

An increasing number of studies show that *CDC20* (cell division cycle 20) plays a role in tumor pathogenesis. In many types of tumors such as breast cancer, pancreatic cancer, and prostate cancer, *CDC20* is highly expressed.[11–13] Mainly through the activation of APC, CDC20 forms an E3 ubiquitin ligase complex called the APC complex (APC^{Cdc20}) to degrade its downstream substrates, regulate the mitogenesis

cycle, and promote apoptosis.[11–14] APC^{Cdc20} regulates the activity of downstream pluripotency-related transcription factor SOX2, which promotes the invasion and renewal of glioma stem cells.[13] Of note, the *CDC20* short interfering (si)RNA that knocks down CDC20 expression inhibits the growth of solid melanoma tumor.[14] Therefore, CDC20 is likely involved in the pathogenesis of melanoma. However, whether it activates the stem cells of melanoma is still unclear.

GNB2 (G protein subunit beta 2) is a member of the Gβ protein family. GNB2 and its related family member GNB1 confer cytokine-independent growth and activate the canonical G protein signaling.[15] G proteins and their downstream signaling targets are involved in the initiation and progression of some cancers, resulting in aberrant cell growth and reduced survival, largely by activating the AKT/mTOR,
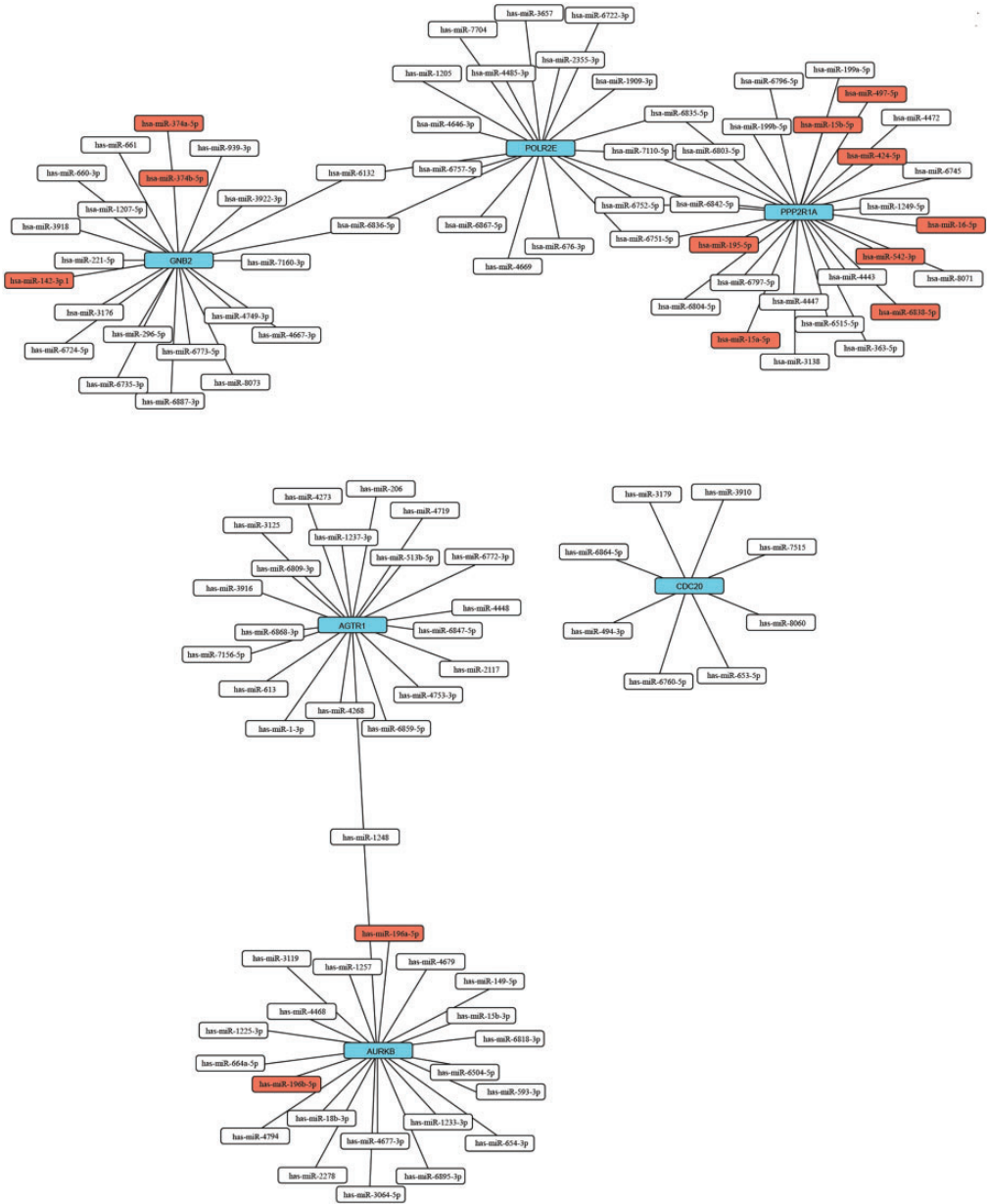
Figure 4. miRNA-hub gene network. Blue boxes represent hub genes, red boxes represent conserved miRNA, and white boxes represent poorly conserved miRNA. Straight lines indicate a regulatory relationship between miRNA and gene. miRNA, microRNA.

MAPK, and Hippo signaling pathways.[16] However, the role of GNB1 in the pathogenesis of melanoma is still not clear. Expression of *GNB2* K78E in A375 melanoma cells that harbor the *BRAF* V600E mutation was shown to confer resistance to vemurafenib.[15] This suggests that GNB2 may play an important role in the
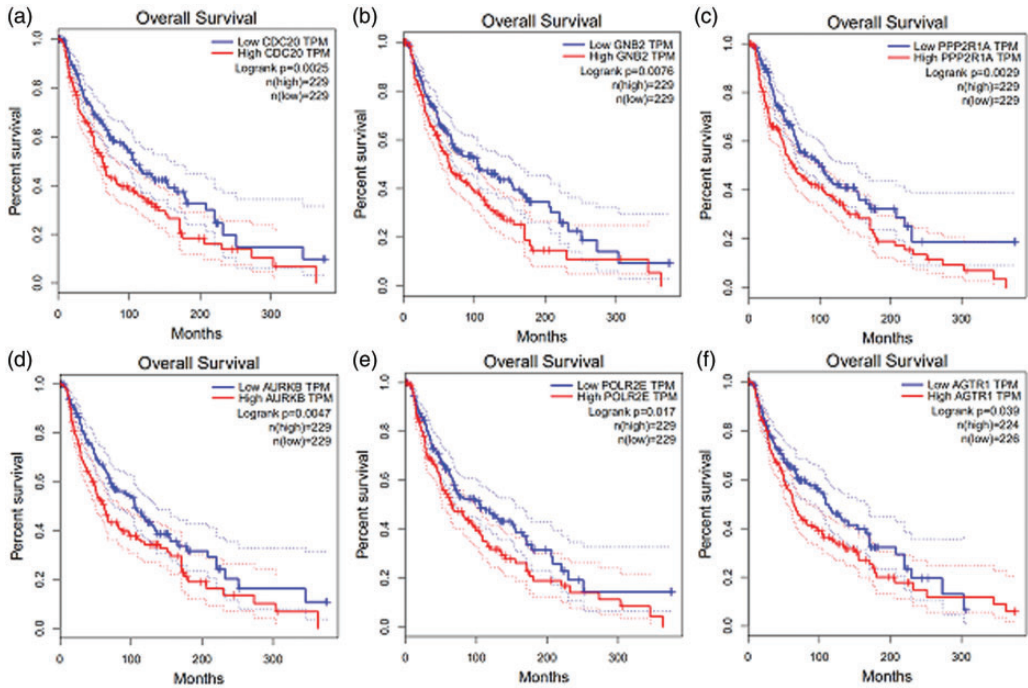
Figure 5. Prognostic value (overall survival) of six genes: *CDC20* (a), *GNB2* (b), *PPP2R1A* (c), *AURKB* (d), *POLR2E* (e), and *AGTR1* (f) in patients with cutaneous melanoma. HR, hazard ratio; CI, confidence interval; TPM, transcripts per million.

pathogenesis and drug resistance of melanoma.

PPP2R1A (protein phosphatase 2 scaffold subunit A alpha) is a scaffolding subunit of protein phosphatase 2A (PP2A), one of four major serine/threonine protein phosphatases.[17] Mutations in *PPP2R1A* occur in breast cancer, lung cancer, and melanoma.[17] PPP2A1A may mediate the survival and resistance to apoptosis of the type B malignant melanoma cell lines.[18] PPP2R1A has also been reported to promote the metastasis of melanoma cells through the interaction of tumor cells and lymphatic endothelial cells.[19] Therefore, PPP2R1A is a potential target associated with the melanoma process.

AURKB (Aurora B kinase) is a chromosomal passenger protein regulating early mitotic stage transition from prophase to metaphase.[20] *AURKB* is overexpressed in a variety of tumors including melanoma.[20] Studies have reported that AURKA controls proliferation, epithelial–mesenchymal transition (EMT), metastasis, and self-renewal capacity of cancer stem cell (CSC), and regulates the cell cycle and survival of cancer cells.[20] In melanoma cells, studies have found that Aurora kinase promotes melanocyte proliferation and reduces apoptosis.[21] Therefore, AURKB is a valuable therapeutic target for melanoma, but its role in melanoma needs further study.

The *POLR2E* gene encodes the fifth largest subunit of RNA polymerase II (RNA polymerase II subunit E), which is responsible for synthesis of messenger RNA in eukaryotes.[22] Meta-analysis results have revealed that POLR2E is associated with the risk of esophageal cancer.[22]

The relationship between POLR2E and melanoma has yet to be established. Our study suggests that *POLR2E* is likely a valuable gene to be further studied in melanoma.

AGTR1 (angiotensin II receptor type 1) is a subtype of the renin-angiotensin system (RAS)[23] and has tumor suppressive function in melanoma. Methylation of an *AGTR1* CpG island is a biomarker of metastatic melanoma[23] and could be used as a new target for gene therapy.

In conclusion, our analysis identified several DEGs associated with the development, progression, and prognosis of melanoma. A total of 1127 DEGs and 10 hub genes were found this study. Based on their high connectivity in the PPI network, *CDC20*, *GNB2*, *PPP2R1A*, *AURKB*, *POLR2E*, and *AGTR1* might be the core genes of melanoma. Further studies are needed to further validate the functions of these genes, including in vitro and in vivo expression and functional analysis.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

ORCID iD

Jing Liu ⓘⒹ https://orcid.org/0000-0002-6805-8503

References

1. Berwick M, Buller DB, Cust A, et al. Melanoma epidemiology and prevention. *Cancer Treat Res* 2016; 167: 17–49.
2. Ribas A, Hamid O, Daud A, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA* 2016; 315: 1600–1609.
3. Ascierto PA, Minor D, Ribas A, et al. Phase II trial (BREAK-2) of the BRAF inhibitor dabrafenib (GSK2118436) in patients with metastatic melanoma. *J Clin Oncol* 2013; 31: 3205–3211.
4. Guo J, Qin S, Liang J, et al. Chinese guidelines on the diagnosis and treatment of melanoma (2015 edition). *Chin Clin Oncol* 2016; 5: 57.
5. Song Z, Lin J, Sun Z, et al. RNAi-mediated downregulation of CDKL1 inhibits growth and colony-formation ability, promotes apoptosis of human melanoma cells. *J Dermatol Sci* 2015; 79: 57–63.
6. Liu P, Hu Y, Ma L, et al. miR-425 inhibits melanoma metastasis through repression of PI3K-Akt pathway by targeting IGF-1. *Biomed Pharmacother* 2015; 75: 51–57.
7. Kubic JD, Lui JW, Little EC, et al. PAX3 and FOXD3 promote CXCR4 expression in melanoma. *J Biol Chem* 2015; 290: 21901–21914.
8. Kanehisa M and Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* 2000; 28: 27–30.
9. Huang da W, Sherman BT and Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 2009; 4: 44–57.
10. Szklarczyk D, Franceschini A, Wyder S, et al. STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* 2015; 43: D447–D452.
11. Mao Y, Li K, Lu L, et al. Overexpression of Cdc20 in clinically localized prostate cancer: relation to high Gleason score and biochemical recurrence after laparoscopic radical prostatectomy. *Cancer Biomark* 2016; 16: 351–358.
12. Chang DZ, Ma Y, Ji B, et al. Increased CDC20 expression is associated with

pancreatic ductal adenocarcinoma differentiation and progression. *J Hematol Oncol* 2012; 5: 15.

13. Karra H, Repo H, Ahonen I, et al. Cdc20 and securin overexpression predict short-term breast cancer survival. *Br J Cancer* 2014; 110: 2905–2913.

14. Mukherjee A, Bhattacharyya J, Sagar MV, et al. Liposomally encapsulated CDC20 siRNA inhibits both solid melanoma tumor growth and spontaneous growth of intravenously injected melanoma cells on mouse lung. *Drug Deliv Transl Res* 2013; 3: 224–234.

15. Yoda A, Adelmant G, Tamburini J, et al. Mutations in G protein beta subunits promote transformation and kinase inhibitor resistance. *Nat Med* 2015; 21: 71–75.

16. O'Hayre M, Degese MS and Gutkind JS. Novel insights into G protein and G protein-coupled receptor signaling in cancer. *Curr Opin Cell Biol* 2014; 27: 126–135.

17. Calin GA, di Iasio MG, Caprini E, et al. Low frequency of alterations of the alpha (PPP2R1A) and beta (PPP2R1B) isoforms of the subunit A of the serine-threonine phosphatase 2A in human neoplasms. *Oncogene* 2000; 19: 1191–1195.

18. Su DM, Zhang Q, Wang X, et al. Two types of human malignant melanoma cell lines revealed by expression patterns of mitochondrial and survival-apoptosis genes: implications for malignant melanoma therapy. *Mol Cancer Ther* 2009; 8: 1292–1304.

19. Christianson DR, Dobroff AS, Proneth B, et al. Ligand-directed targeting of lymphatic vessels uncovers mechanistic insights in melanoma metastasis. *Proc Natl Acad Sci U S A* 2015; 112: 2521–2526.

20. Tang A, Gao K, Chu L, et al. Aurora kinases: novel therapy targets in cancers. *Oncotarget* 2017; 8: 23937–23954.

21. Bonet C, Giuliano S, Ohanna M, et al. Aurora B is regulated by the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) signaling pathway and is a valuable potential target in melanoma cells. *J Biol Chem* 2012; 287: 29887–29898.

22. Chu H, Chen Y, Yuan Q, et al. The HOTAIR, PRNCR1 and POLR2E polymorphisms are associated with cancer risk: a meta-analysis. *Oncotarget* 2017; 8: 43271–43283.

23. Renziehausen A, Wang H, Rao B, et al. The renin angiotensin system (RAS) mediates bifunctional growth regulation in melanoma and is a novel target for therapeutic intervention. *Oncogene* 2019; 38: 2320–2336.