Exploration and Validation of Metastasis-associated Genes for Skin Cutaneous Melanoma

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Abstract

Background: Skin cutaneous melanoma is a malignant and highly metastatic skin tumor, and its morbidity and mortality are still rising worldwide. However, the molecular mechanisms that promote melanoma metastasis are unclear.

Methods: Two datasets (GSE15605 and GSE46517) were retrieved to identify the differentially expressed genes (DEGs), including 23 normal skin tissues (N), 77 primary melanoma tissues (T) and 85 metastatic melanoma tissues (M). Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment were performed to explore the functions of the DEGs. The protein–protein interaction (PPI) network was constructed using the STRING tool and Cytoscape software. We used the cytoHubba plugin of Cytoscape to identify the most significant hub genes by five topological analyses (Degree, Bottleneck, MCC, MNC, and EPC). Hub gene expression was validated using the UALCAN website. Clinical relevance was investigated using The Cancer Genome Atlas (TCGA) resources. Finally, we explored the association between metastasis-associated genes and immune infiltrates through the Tumor Immune Estimation Resource (TIMER) database and performed drug-gene interaction analysis using the Drug-Gene Interaction database.

Results: A total of 294 specific genes were related to melanoma metastasis and were mainly involved in the positive regulation of locomotion, mitotic cell cycle process, and epithelial cell differentiation. Four hub genes (CDK1, FOXM1, KIF11, and RFC4) were identified from the cytoHubba plugin of Cytoscape. CDK1 was significantly upregulated in metastatic melanoma compared with primary melanoma, and high expression of CDK1 was positively correlated with poor prognosis. We found that CDK1 expression correlated positively with the infiltration levels of macrophage cells ($Rho = -0.164$, $P = 2.02e-03$) and neutrophil cells ($Rho = 0.269$, $P = 2.72e-07$) in SKCM metastasis. In addition, we identified that CDK1 had a close interaction with 10 antitumor drugs.

Conclusions: CDK1 was identified as a hub gene involved in the progression of melanoma metastasis and may be regarded as a therapeutic target for melanoma patients to improve prognosis and prevent metastasis in the future.

1 Introduction

Skin cutaneous melanoma (SKCM) is an aggressive and highly metastatic skin tumor. The 5-year relative survival rate for localized melanoma is 99%, but it is only 25% once tumor metastasis occurs [1]. The American Cancer Society estimated that there were approximately 100,350 newly diagnosed melanoma patients in 2020 in the United States, and the melanoma incidence rate has significantly increased in the past decade worldwide [1, 2]. Melanoma has become a serious human health issue, bringing a huge economic burden to society. When melanoma is diagnosed at an early stage, surgical resection is the most effective treatment strategy [3]. However, patients suffering from metastatic melanoma have a poor prognosis; therefore, it is necessary to identify new diagnostic biomarkers of melanoma metastasis.

Melanoma is one of the most sensitive tumors to immune regulation [4]. Recently, immunotherapy has achieved durable improvements in survival in patients with advanced stage and metastatic melanoma [5, 6]. A recent study showed that the 5-year overall survival rate for untreated patients with advanced patients or recurrent malignant melanoma treated with nivolumab was 26.1% [7]. Despite the effective therapeutic effect obtained with currently approved treatment strategies, due to the heterogeneous nature and high mutation rate of melanoma [4, 8], primary and secondary resistance and the relatively low response rate of immunotherapy for metastatic melanoma remain challenging [9]. Therefore, exploring the exact molecular mechanisms that promote melanoma metastasis and discovering novel immunotherapeutic targets for melanoma are crucial.

In this study, we compared mRNA expression between groups of normal skin (N), primary melanoma (T) and melanoma metastasis (M) samples from the Gene Expression Omnibus (GEO) database. The differentially expressed genes (DEGs) were subjected to gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology and protein–protein interaction (PPI) network analyses to determine their functions. We further validated the expression levels and verified the clinical significance of the identified genes using clinical samples. Finally, immune infiltration and target drugs prediction were further performed to elucidate the potential function of the hub genes. Our data provide novel information and help further understand the metastasis cascade of melanoma.

2 Materials And Methods

2.1 Data collection and processing

Two gene expression profiles of SKCM, namely GSE15605 [10] and GSE46517 [11], were retrieved from the GEO database (<http://www.ncbi.nlm.nih.gov/geo>). Among them, GSE15605 consisted of 16 normal skin tissues (N), 46 primary melanoma tissues (T) and 12 metastatic melanoma tissues (M), and GSE46517 contained 7 normal skin tissues, 31 primary tissues and 73 metastatic tissues. The mRNA expression profiles of GSE15605 and GSE46517 were detected by using the GPL570 platform (Affymetrix Human Genome U133 Plus 2.0 Array). GEO2R is an interactive web tool (<http://www.ncbi.nlm.nih.gov/geo/geo2r>) using the 'limma' package of R to screen for differentially expressed mRNAs in T vs. N and M vs. N. $P < 0.05$ and $|\log_2FC| > 1$ were set as the cutoff criteria for identifying DEGs.

2.2 GO and KEGG pathway analysis

GO analysis and KEGG enrichment were performed on the DEGs, and the results were visualized using the R package. A P value < 0.05 was set as the cutoff criterion.

2.3 PPI network construction and hub gene selection and analyses

The Search Tool for the Retrieval of Interacting Genes (STRING) [12] (<http://string-db.org>) (version 10.0) was used to construct the PPI network of 294 DEGs. Those with a score ≥ 0.7 were used as the cutoff criterion [12]. Then, the string interactions were imported into Cytoscape [13] (<http://www.cytoscape.org>) (version 3.7.2) for visualization. Specifically, we applied the Cytoscape plugin CytoHubba [14] to identify hub genes via five models: maximal clique centrality (MCC), maximum neighborhood component (MNC), Degree, edge percolated component (EPC) and Bottleneck.

2.4 Validation of hub gene expression

We analyzed the mRNA expression of hub genes using TCGA data, which were obtained from the UALCAN website (<http://ualcan.path.uab.edu/analysis.html>) [15]. A total of 473 samples, including 1 normal tissue, 104 primary melanomas and 368 metastatic melanomas, were contained. $P < 0.05$ was statistically significant. Protein expression of hub genes was evaluated in primary melanoma and metastasis tissues using the Human Protein Atlas tool (<https://www.proteinatlas.org/>) [16].

2.5 Survival Analysis of hub genes by TCGA.

Then, based on the fragments per kilobase million value of each gene, melanoma patients were classified into two expression groups (high expression and low expression), and the correlation between the expression level and patient survival was examined by Gene Expression Profiling Interactive Analysis (GEPIA) (<http://gepia.cancer-pku.cn/index.html>). The overall survival (OS) curve of hub genes was also analyzed by the UALCAN website, and $P < 0.05$ was considered statistically significant.

2.6 Immune infiltration analysis

The relationship between metastasis-associated gene expression and the abundance of immune infiltration in SKCM metastasis was evaluated using the Tumor Immune Estimation Resource (TIMER) [17]. TIMER included six immune cells: B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells. $P < 0.05$ was identified to be significant.

2.7 Drug-gene interaction networks analysis

The potential metastasis-associated genes were supposed to be promising drug targets for searching drugs through the Drug-Gene Interaction database (DGIdb, version 4.2.0-sha1 afd9f30b, <http://www.dgldb.org/>) [18], which helps to predict drug-gene interaction networks, with the

parameters set as follows: preset filters: antineoplastic; all the default. After the prediction of drug-gene pairs related to the metastasis-associated genes, the network map was then formed by Cytoscape.

3 Results

3.1 Screening for DEGs

To identify the metastasis-associated gene signature in melanoma, we compared mRNA expression levels in T vs. N and M vs. N. $|\log_2FC| > 1$ and $P < 0.05$ were defined as statistically meaningful cutoff points. After analyzing the transcriptomic changes of T vs. N, a total of 3267 DEGs, including 1444 upregulated and 1823 downregulated transcription factors, were screened in GSE15605, and a total of 1127 DEGs, including 527 upregulated and 600 downregulated transcription factors, were identified in GSE46517 (Fig. 1a, c). A total of 5775 DEGs, 3017 upregulated and 2758 downregulated in M vs. N in GSE15605. The volcano plot of DEG distribution presented 2016 DEGs, including 952 upregulated and 1064 downregulated DEGs, when comparing M with N in GSE46517 (Fig. 1b, d). As shown in the Venn diagram (Fig. 1e), a total of 1355 genes overlapped between the two metastasis-associated datasets, which might play an important role in melanoma metastasis, but except for 1061 genes related to tumor development, 294 genes were specific for melanoma metastasis.

3.2 Functional enrichment analysis for DEGs

All 294 screened DEGs were subjected to GO and KEGG pathway analysis by the R package. In GO biological process analysis, DEGs were mainly concentrated on positive regulation of locomotion, mitotic cell cycle process, epithelial cell differentiation, and actin filament-based process. The results of KEGG pathway analysis revealed that DEGs were dominant in pathways in cancer, microRNAs in cancer, phospholipase D signaling pathway, and purine metabolism (Fig. 2).

3.3 PPI network analysis and hub Gene screening

The STRING database was used to draw the PPI network diagram of the DEGs. DEGs with a combined score ≥ 0.7 were eligible for constructing the relational network. We ranked the top 20 genes of the whole network based on the Cytoscape plugin CytoHubba models: Degree, MCC, MNC, EPC and Bottleneck (Fig. 3a-e). Venn analysis was performed to obtain the intersection of these genes. Notably, the top 20 genes from topological analysis algorithms included four hub genes: CDK1, FOXM1, KIF11, and RFC4, which may play important roles in the development of metastatic melanoma (Fig. 3f).

3.4 Validation of hub genes expression

To further verify previously defined hub genes, we obtained expression data of 473 TCGA-SKCM samples, including 1 normal tissue, 104 primary melanomas and 368 metastatic tissue samples from the UALCAN database. The results showed that three hub genes (CDK1, KIF11 and RFC4) were significantly upregulated in metastatic melanoma compared with primary melanoma (Fig. 4a-d). Significant differences are indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. FOXM1 expression was not significantly different between primary melanoma and metastatic melanoma tissues ($P > 0.05$). Protein expression levels of four hub genes were compared using the Human Protein Atlas database. Typical immunohistochemistry images (<https://www.proteinatlas.org/>) for the protein expression of the hub gene in primary melanoma and metastatic melanoma tissue are shown in Figure 4e-h.

3.5 Identification of the clinical prognostic value of hub genes in SKCM.

To estimate the influence of hub gene expression on the prognosis of SKCM, the TCGA SKCM dataset was used to identify hub genes associated with overall survival (OS) and disease-free survival (DFS) by GEPIA. As shown in Figure 5, high mRNA expression levels of two hub genes (CDK1: OS $P = 0.037$; FOXM1: OS $P = 9.7\text{e-}06$) were significantly correlated with poor prognosis. The expression of KIF11 and RFC4 had no correlation with OS and DFS. To further confirm the results, the expression of four hub genes with OS were analyzed by UALCAN database. The results indicated that the high expression of CDK1 and FOXM1 was positively correlated with poor prognosis (CDK1: OS $P = 0.047$; FOXM1: OS $P = 0.00043$) (Fig. 1S).

3.6 Immune infiltration analysis

We analyzed the correlations between CDK1 and FOXM1 expression and the immune infiltration levels of six immune cells (CD8+ T cells, CD4+ T cells, B cells, neutrophils, macrophages and myeloid dendritic cells) in the SKCM metastasis microenvironment using TIMER. $P \leq 0.05$ was considered as significance. We found that FOXM1 expression was positively correlated with the infiltration degree of myeloid dendritic cells (Rho = 0.124, $P = 1.94\text{e-}02$) (Fig 6a, S2). CDK1 expression was associated with infiltrating levels of macrophage cells (Rho = -0.164, $P = 2.02\text{e-}03$) and neutrophil cells (Rho = 0.269, $P = 2.72\text{e-}07$) (Fig. 6b). The expression of CDK1 was not associated with the immune infiltration levels of CD8+ T cells, CD4+ T cells, B cells or dendritic cells (Fig. S2).

3.7 Drug-gene interaction networks analysis

DGIdb was used to predict the drugs that potentially interacted with the CDK1 and FOXM1 genes. We identified 10 drugs that interacted with CDK1; however, there was no drug that interacted with FOXM1, as shown in Figure 6c. These results may reveal the therapeutic targets related to metastatic melanoma.

4 Discussion

Tumor metastasis is the most common recurrence mode and cause of death in SKCM, which seriously affects patient survival and prognosis. Thus, it is urgent to better understand the molecular mechanisms of metastasis and explore effective indicators for monitoring this pathophysiological process.

In the present study, we identified 294 genes that were closely related to melanoma metastasis. GO analysis showed that DEGs were mainly enriched in the positive regulation of locomotion, mitotic cell cycle process, epithelial cell differentiation, and actin filament-based process. The above biological processes are mainly related to cell movement and cell proliferation. We speculate that DEGs induced melanoma cell metastasis by promoting cell migration and cell proliferation. KEGG enrichment analysis revealed that DEGs were mainly enriched pathways in cancer, microRNAs in cancer, phospholipase D signaling pathway, and purine metabolism. Phospholipase D is an enzyme that catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid. Studies have found that the phospholipase D signaling pathway is involved in lung cancer-mediated bone metastasis[19], and phospholipase D isoform 1 promotes tumor invasion of [bladder cancer](#) by regulating MMP-13 expression[20]. The role of the phospholipase D signaling pathway in melanoma metastasis is worth further exploration.

Next, we screened 4 specific metastasis-associated genes by conducting a PPI network and analyzed their prognostic value through the TCGA database. The results suggest that CDK1 and FOXM1 may be favorable predictive factors for melanoma patient survival. FOXM1 is a regulator of myriad biological processes, including cell proliferation, cell cycle progression, cell differentiation, DNA damage repair, tissue homeostasis, angiogenesis and apoptosis, and has been suggested to be a key player in tumorigenesis [21]. Moreover, FOXM1 is a known downstream factor of the Akt signaling cascade [22, 23], and inactivation of the Akt/FOXM1 signaling pathway may suppress the proliferation and metastasis of gastric cancer cells [24]. In our present study, we found that FOXM1 expression was not significantly different between metastatic melanoma tissues and primary melanoma tissues, and FOXM1 seemed not to be a potential metastasis-associated biomarker for melanoma. We therefore further researched the CDK1 gene, which is specifically involved in the metastasis process of melanoma and predicts a poor prognosis.

CDK1, namely, cyclin-dependent kinase 1, is a serine/threonine-like protein kinase and plays critical roles in the regulation of the cell cycle and cell proliferation. Previous studies have reported that CDK1 forms a complex with cyclin A/B that is involved in the regulation of mitosis, G2/M checkpoint maintenance, execution of apoptosis, maintenance of pluripotency and genomic stability [25]. Furthermore, CDK1 has been revealed to be overexpressed in various malignancies. High expression of CDK1 may lead to poor clinical prognosis, and inhibition of CDK1 enhances 5-Fu sensitivity in colorectal cancer [26]. Z. Huang *et al.* reported that CDK1 positively regulated the stemness of lung cancer cells in a Sox2-dependent manner, and inhibition of CDK1 enhanced chemotherapeutic sensitivity in lung cancer [27]. Recently, CDK1-mediated phosphorylation of TFCP2L1 has been shown to be required for stem cell pluripotency

and bladder carcinogenesis[28]. CDK1 interacts with Sox2 and promotes tumor initiation in human melanoma[29]. The role of CDK1 in melanoma metastasis has not been reported previously.

The tumor microenvironment (TME) is composed of immune cells, mesenchymal cells, endothelial cells, inflammatory mediators and extracellular matrix (ECM) molecules [30, 31]. The composition and abundance of immune cells in the tumor microenvironment strongly influence the progression of tumors and the effect of immunotherapy [32-34]. We found that the expression levels of CDK1 were correlated with immune cell infiltration. We speculate that CDK1 is involved in the process of melanoma metastasis by regulating the function of the tumor microenvironment. Therefore, the function and pathway of CDK-mediated immune cell-infiltration need to be further investigated.

Currently, according to the genotype and stage of melanoma, targeted therapy, such as BRAF inhibitors and MEK inhibitors, has been used as first-line treatment or adjuvant therapy for patients [35]. In 2011, the FDA approved the first targeted therapeutic drug for advanced BRAF-mutant melanoma, vemurafenib [36]. In this study, ten drugs were identified, that may serve as potential therapeutic targets. Rucaparib, as a PAPP inhibitor, has been approved by the FDA for the clinical treatment of ovarian cancer [37]. In a phase II study, temozolomide (150–200 mg/m²/day) was safely given with rucaparib, increasing progression-free survival over historical controls in patients with advanced metastatic melanoma[38]. Dinaciclib induces p53 expression while simultaneously downregulating the expression of the antiapoptotic factors Mcl-1 and XIAP in melanoma cell lines [39]. The small-molecule drug AT-7519 diminished MDM4 levels and activated p53 in the A375 melanoma cell line [40]. Other drugs have not been reported in the literature for the treatment of melanoma metastasis.

The main limitation of our study was only the analysis of bioinformatics, so it was urgent to carry out cytological experiments, animal experiments, and drug trials to verify these hub genes in melanoma metastasis.

5 Conclusions

In summary, 294 DEGs between the primary and metastatic skin cutaneous melanoma samples were screened, and four hub genes, CDK1, FOXM1, KIF11, and RFC4, were identified that may be associated with the metastasis of melanoma. UALCAN suggested that CDK1 was significantly upregulated in metastatic melanoma compared with primary melanoma and that high expression of CDK1 was positively correlated with poor prognosis. Moreover, CDK1 plays an important role in the microenvironment of metastatic melanoma by regulating the tumor infiltration of immune cells. Ten candidate small molecule antitumor drugs were selected. Further studies are needed to investigate these metastasis-associated genes as therapeutic targets.

Abbreviations

SKCM: Skin cutaneous melanoma; GEO: Gene Expression Omnibus;

DEG: differentially expressed gene analyses; GO: gene ontology;

KEGG: Kyoto Encyclopedia of Genes and Genomes; PPI: protein-protein interaction;

GEPIA: Gene Expression Profiling Interactive Analysis; DGIdb: Drug-Gene Interaction database TIMER: Tumor Immune Estimation Resource; OS: overall survival; (DFS) disease-free survival

TME: tumor microenvironment;

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The data used to support the findings of this study were available from the Gene Expression Omnibus (<http://www.ncbi.nlm.nih.gov/geo>) and Cancer Genome Atlas database (<https://cancergenome.nih.gov>).

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' Contributions

LPZ designed and supervised study. HL, LGJ and YH analyzed the data, manufactured the figures, and wrote of the manuscript. TZ, YNS and LPZ revised and edited the manuscript. All authors have read and approved the final manuscript.

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Figures

Figure 1

Identification of specific genes associated with metastasis in melanoma. (A) Differentially expressed genes (DEGs) were screened by volcano plot comparing 16 normal skin (N), 46 primary melanoma (T) and 12 metastatic melanoma tissues (M) from GSE15605. (B) DEGs were screened by volcano plot when comparing 7 N samples, 31 T samples and 73 M samples in GSE46517. (E) Venn diagram for overlapping DEGs in 4 microarray datasets. $|\log_2 \text{FC}| > 1$ and adj. $P < 0.05$ were set as the cutoff criterion.

Figure 2

GO and KEGG pathway enrichment analysis of DEGs. (A) Top ten enriched biological processes for the DEGs. (B) Top ten enriched KEGG pathways for the DEGs.

Figure 3

Identification of hub genes. (A-E) The hub genes were identified using five topological analysis methods (A) Degree, (B) Bottleneck (C) MCC (D) MNC, and (E) EPC with cytoHubba (E) A Venn diagram showed that four hub genes were identified.

Figure 4

Box plot of hub gene expression in primary and metastatic melanoma samples. (A) CDK1 (B) FOXM1 (C) KIF11 (D) RFC4. *P < 0.05; **P < 0.01; ***P < 0.001. (E) Representative protein expression of eight genes in primary melanoma and metastasis tissues using the human protein atlas tool in pancreatic cancer. Data were obtained from the Human Protein Atlas database.

Figure 5

Association of hub gene expression levels and prognosis of SKCM based on the TCGA SKCM datasets by GEPIA. $P < 0.05$ was considered statistically significant.

Figure 6

Hub gene analysis. (A) Correlation analyses of FOXM1 expression with the infiltration degree of myeloid dendritic cells in SKCM metastasis through TIMER database. (B) Correlation analyses of CDK1 expression and immune infiltrates (neutrophils and macrophages) in SKCM metastasis through TIMER database. (C) Drug-hub gene interaction network. Blue nodes represented the drug. The line represents the interaction relationship between CDK1 and the drug.

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