Identification of key candidate genes involved in melanoma metastasis

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Abstract. Metastasis is the most lethal stage of cancer progression. The present study aimed to investigate the underlying molecular mechanisms of melanoma metastasis using bioinformatics. Using the microarray dataset GSE8401 from the Gene Expression Omnibus database, which included 52 biopsy specimens from patients with melanoma metastasis and 31 biopsy specimens from patients with primary melanoma, differentially expressed genes (DEGs) were identified, subsequent to data preprocessing with the affy package, followed by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. A protein-protein interaction (PPI) network was constructed. Mutated genes were analyzed with 80 mutated cases with melanoma from The Cancer Genome Atlas. The overall survival of key candidate DEGs, which were within a filtering of degree >30 criteria in the PPI network and involved three or more KEGG signaling pathways, and genes with a high mutation frequency were delineated. The expression analysis of key candidate DEGs, mutant genes and their associated genes were performed on UALCAN. Of the 1,187 DEGs obtained, 505 were upregulated and 682 were downregulated. 'Extracellular exosome' processes, the 'amoebiasis' pathway, the 'ECM-receptor interaction' pathway and the 'focal adhesion' signaling pathway were significantly enriched and identified as important processes or signaling pathways. The overall survival analysis of phosphoinositide-3-kinase regulator subunit 3 (*PIK3R3*), centromere protein M (*CENPM*), aurora kinase A (*AURKA*), laminin subunit α 1 (*LAMA1*), proliferating cell nuclear antigen (*PCNA*), adenylate cyclase 1 (*ADCY1*), BUB1 mitotic checkpoint serine/threonine kinase (*BUB1*), NDC80 kinetochore complex component (*NDC80*) and protein kinase C α (*PRKCA*) in DEGs was statistically significant. Mutation gene analysis identified that BRCA1-associated protein 1 (*BAP1*) had a higher mutation frequency and survival analysis, and its associated genes in the *BAP1*-associated PPI network, including ASXL transcriptional regulator 1 (*ASXL1*), proteasome 26S subunit, non-ATPase 3 (*PSMD3*), proteasome 26S subunit, non ATPase 11 (*PSMD11*) and ubiquitin C (*UBC*), were statistically significantly associated with the overall survival of patients with melanoma. The expression levels of *PRKCA*, *BUB1*, *BAP1* and *ASXL1* were significantly different between primary melanoma and metastatic melanoma. Based on the present study, 'extracellular exosome' processes, 'amoebiasis' pathways, 'ECM-receptor interaction' pathways and 'focal adhesion' signaling pathways may be important in the formation of metastases from melanoma. The involved genes, including *PIK3R3*, *CENPM*, *AURKA*, *LAMA1*, *PCNA*, *ADCY1*, *BUB1*, *NDC80* and *PRKCA*, and mutation associated genes, including *BAP1*, *ASXL1*, *PSMD3*, *PSMD11* and *UBC*, may serve important roles in metastases of melanoma.

Introduction

Melanoma is a common malignancy and has one of the poorest prognoses due to its excessive aggression and metastasis (1). In recent years, the incidence of melanoma has increased significantly and the survival rate of patients with melanoma remains poor (2). As metastasis is the most lethal stage of melanoma progression, identifying its underlying molecular mechanisms and determining its molecular biomarkers is important.

Microarray analysis is able to quickly identify all of the genes expressed at any one time-point and is particularly suited to screen for differentially expressed genes (DEGs) (3). Recently, microarray analysis has been widely applied and has produced large amounts of microarray data, which are deposited in public databases. It may be beneficial for further research to integrate and reanalyze this data (4).

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In a previous study, the microarray dataset GSE8401 generated by Xu *et al* (5) was used to validate a 'metastasis aggressiveness gene expression signature' derived from human melanoma cells, selected based on their metastatic potential in an immunodeficient mouse metastasis model, and their correlation with the aggressiveness of melanoma metastases in human patients. However, an integral bioinformatics analysis combined with an expression profile analysis has not been yet been conducted, to the best of the authors' knowledge.

In the present study, the DEGs from the GSE8401 dataset were identified, subsequent to data preprocessing with the affy package, followed by Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. A protein-protein interaction (PPI) network was constructed. Mutated genes were analyzed with 80 mutated cases with melanoma from The Cancer Genome Atlas (TCGA). The overall survival of key candidate DEGs, which were within a filtering of degree >30 criteria in the PPI network and involved three or more KEGG signaling pathways, and genes with a high mutation frequency were delineated. The expression analysis of key candidate DEGs, mutant genes and their associated genes were performed on UALCAN. Of the 1,187 DEGs obtained, 505 were upregulated and 682 were downregulated. 'Extracellular exosome' processes, the 'amoebiasis' pathway, the 'ECM-receptor interaction' pathway and the 'focal adhesion' signaling pathway were significantly enriched and identified as important processes or signaling pathways. The overall survival analysis of phosphoinositide-3-kinase regulator subunit 3 (*PIK3R3*), centromere protein M (*CENPM*), aurora kinase A (*AURKA*), laminin subunit α 1 (*LAMA1*), proliferating cell nuclear antigen (*PCNA*), adenylate cyclase 1 (*ADCY1*), BUB1 mitotic checkpoint serine/threonine kinase (*BUB1*), NDC80, kinetochore complex component (*NDC80*) and protein kinase C α (*PRKCA*) in DEGs was statistically significant. Mutation gene analysis identified that BRCA1-associated protein 1 (*BAP1*) had a higher mutation frequency and survival analysis, and its associated genes in the *BAP1*-associated PPI network, including ASXL transcriptional regulator 1 (*ASXL1*), proteasome 26S subunit, non-ATPase 3 (*PSMD3*), proteasome 26S subunit, non ATPase 11 (*PSMD11*) and ubiquitin C (*UBC*), were statistically significantly associated with the overall survival of patients with melanoma. The expression levels of *PRKCA*, *BUB1*, *BAP1* and *ASXL1* were significantly different between primary melanoma and metastatic melanoma. Based on the present study, 'extracellular exosome' processes, 'amoebiasis' pathways, 'ECM-receptor interaction' pathways and 'focal adhesion' signaling pathways may be important in the formation of metastases from melanoma. The involved genes, including *PIK3R3, CENPM, AURKA, LAMA1, PCNA, ADCY1, BUB1, NDC80* and *PRKCA*, and mutation associated genes, including *BAP1, ASXL1, PSMD3, PSMD11* and *UBC*, may serve important roles in metastases of melanoma.

Materials and methods

Affymetrix microarray data and clinical information. The gene expression profile dataset GSE8401 was extracted from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/) (6). The GSE8401 dataset was deposited by Xu *et al* (5), including 52 biopsy specimens from patients with melanoma metastasis and 31 biopsy specimens from patients with primary melanoma, and was based on the platform of the GPL96 (HG-U133A) Affymetrix Human Genome U133A Array (Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA). These 83 fresh melanoma biopsies from patients undergoing surgery were collected between 1992 and 2001 as a part of the diagnostic or therapeutic strategy. Immediately following surgery, half of each specimen was fixed in formalin and processed for routine histology, and the other half was immediately snap-frozen and stored in liquid nitrogen until use for RNA extraction. Histopathological diagnosis of each tissue specimen was performed independently by two histopathologists. All patient specimens were collected and used, according to the approval by the institutional ethics committee and written informed consent was obtained, according to the ethical standards in the 1964 Declaration of Helsinki.

Data preprocessing and DEG screening. The downloaded data in the series matrix file was preprocessed using the affy package (version 1.50.0) (7) in R language, including normalization and expression calculation. Annotations to the probes were performed, and probes that were not matched to the gene symbols were excluded. The average expression values were taken if different probes mapped to the same gene. DEGs in patients with melanoma metastasis compared with patients with primary melanoma were analyzed using the limma package (8) in R language. The cut-off threshold was set as $P<0.05$ and $\log_2$ (fold change) >1 or <-1.

GO and pathway enrichment analysis. GO (http://www.geneontology.org/) analysis is commonly used for functional studies of large-scale genomic or transcriptomic data and classifies functions with respect to three criteria: Molecular function (MF), cellular component (CC) and biological process (BP) (9,10). The KEGG (http://www.kegg.jp/) pathway database is widely used for the systematic analysis of gene functions, associating genomic data with higher order functional data (11). The Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.ncifcrf.gov/) is an integrated biological knowledge base with analytical tools used for the systematic and integrative analysis of large gene lists (12). In the present study, GO terms and KEGG signaling pathway enrichment analyses for DEGs were performed using DAVID. The cutoff threshold was set as $P<0.05$.

Integration analysis of the PPI network and the KEGG pathway network. Search Tool for the Retrieval of Interacting Gene/Proteins (STRING; http://www.string-db.org/) is an online database that assesses and integrates PPIs (13). In the present study, DEGs were mapped onto the STRING database for PPI analysis, with a combined score of 0.4 as the parameter setting. The PPI network generated with the DEGs was constructed with Cytoscape software (version 3.4.0) (14,15) and the topology scores of the nodes in the PPI network were analyzed using the CentiScape plugin (version 3.4.0; Parameter setting: Without weight) (16). The key candidate

genes were screened with a filtering of degree >30 criteria in the PPI network. Networks of significantly enriched (P<0.05) KEGG pathways and their associated genes were constructed using the Cytoscape (version 3.4.0) plugin ClueGO (14) and Cluepedia (15). The cross-talk genes were identified with the filtering criteria of association with at least three signaling pathways.

Mutant genes screening. The cBioPortal (http://www.cbioportal.org/) for Cancer Genomics, which provides the visualization, analysis and download of large-scale cancer genomics datasets, was employed for the mutation analysis (17,18). In total, 80 mutated cases were selected from TCGA database (project ID: TCGA_SKCM; https://cancergenome.nih.gov/) to screen for mutated genes (19). The detailed clinical information of the samples is listed in Table I.

Overall survival and expression analysis. Overall survival analyses for key candidate DEGs and mutation associated genes were performed using Gene Expression Profiling Interactive Analysis (http://gepia.cancer-pku.cn/index.html) (20). Briefly, by entering the genes of interest to the website, patients were divided into two groups according to the expression level of gene, and the survival rate was statistically analysed. The hazard ratio (HR) with 95% confidence intervals and log rank P-value were calculated and are presented. The expression analysis of key candidate DEGs and mutation-associated genes in melanoma was performed using UALCAN (http://ualcan.path.uab.edu/analysis.html) (21).

Results

Data normalization and analysis of DEGs. The boxplot demonstrated good normalization of the GSE8401 data (Fig. 1A). In total, 22,283 probes were obtained, among which 1,205 probes were differentially expressed. Following annotation, 1,187 DEGs in patients with melanoma metastasis compared with patients with primary melanoma were identified, including 505 upregulated DEGs and 682 downregulated DEGs (Fig. 1B).

GO and signaling pathway enrichment analysis. GO enrichment analysis for the DEGs was performed. The top 10 most significant GO terms of each group are presented in Fig. 2A. For MF, the DEGs were primarily enriched in 'structural molecule activity' processes. For CC, the DEGs were primarily enriched in 'extracellular exosome' processes and 'extracellular space' processes. For BP, the DEGs were primarily enriched in 'epidermis development' processes, 'keratinocyte differentiation' processes and 'cell adhesion' processes.

The top 20 most significant KEGG pathway terms are presented in Fig. 2B. The genes were primarily enriched in 'amoebiasis', 'ECM-receptor interaction' and 'focal adhesion' signaling pathways.

PPI analysis. Using the STRING online database and Cytoscape software, a total of 447 DEGs of the 1,187 DEGs were mapped onto the PPI network complex (Fig. 3), and 740 of 1,187 DEGs did not fall into the PPI network.

Cross-talk genes and key candidate gene screening. The cross-talk genes were identified with the filtering criteria of association with at least three signaling pathways, based on the KEGG pathway analysis (Fig. 4). The key candidate genes were screened, including *PIK3R3, CENPM, AURKA, LAMA1, PCNA, ADCY1, BUB1*, NDC80 kinetochore complex component *(NDC80)* and *PRKCA*. Among these genes, *PIK3R3, PRKCA, ADCY1* and *LAMA1* were enriched in the 'amoebiasis' signaling pathway. *ADCY1* was additionally involved in 'pancreatic secretion', 'GnRH signaling pathway', 'melanogenesis' and 'pathways in cancer' signaling pathways. The majority of these genes were involved in 'pathways in cancer', 'ECM-receptor interaction' and 'focal adhesion' signaling pathways.

Overall survival analysis of key candidate genes. The survival analysis sourced from TCGA data was performed and the analysis curves demonstrated that the aberrant expressions of the key candidate genes, including *PIK3R3, CENPM, AURKA, LAMA1, PCNA, ADCY1, BUB1, NDC80* and *PRKCA*, were significantly associated with poor overall survival (Fig. 5).

Mutant genes and their associated gene analysis. The mutation analysis of genes was performed on cBioPortal based on 80 patients with melanoma from TCGA database. The results demonstrated that G protein subunit α q, G protein subunit α 11, *BAP1* and slicing factor 3b subunit 1 had the highest mutation frequency (Fig. 6). Only the survival analysis of BAP1 was statistically significant (data not shown).

A *BAP1*-associated PPI network was constructed (Fig. 7), in which the survival analyses of ASXL1, proteasome 26S subunit, non-ATPase 3 (*PSMD3*), proteasome 26S subunit, non ATPase 11 (*PSMD11*) and *UBC* were statistically significant (Fig. 8). Survival analysis was conducted in 40 melanoma cases with metastasis selected from the 80 patients with melanoma in the TCGA database (14 cases with BAP1 mutations and 26 cases without BAP1 mutations) to identify the prognostic value of BAP1 mutations in melanoma cases with metastasis (Fig. 9); information on the cases is provided in Table I.

Discussion

The majority of patients with melanoma are diagnosed at advanced stages and have poor overall survival (22). However, the molecular mechanisms involved in the metastasis and progression of malignant melanoma remain unclear.

In the present study, the raw gene expression data of GSE8401 was obtained from the GEO. The original previous study identified that membrane proteins served an important role in tumor-microenvironment interactions during metastasis (5). However, further investigation is required regarding the clinical value of these genes. In the present study, a comprehensive bioinformatics analysis based on the GSE8401 dataset and TCGA was performed. The results suggested that high expression of *PIK3R3, CENPM, AURKA, LAMA1, PCNA, ADCY1, BUB1, NDC80* and *PRKCA* in patients with melanoma was associated with poor overall survival.

Table I. Information of the cases with metastasis.

A, Deceased patients

Submitter ID	*BAP1* mutation	Tumor stage	Days to mortality
TCGA-WC-A88A	Yes	IV	82
TCGA-WC-A884	Yes	IIIb	123
TCGA-WC-A883	Yes	IIIa	241
TCGA-WC-A87U	Yes	IIIa	581
TCGA-VD-A8KF	No	IIIb	240
TCGA-VD-A8KD	No	IV	114
TCGA-V4-A9EX	No	IIIa	730
TCGA-V4-A9EV	No	IIIa	1,105
TCGA-V4-A9ES	Yes	IIIa	511
TCGA-V4-A9EQ	Yes	IIIa	355
TCGA-V4-A9EI	No	IIIb	389
TCGA-V4-A9E9	Yes	IIIa	1,246
TCGA-V4-A9E8	Yes	IIIa	458
TCGA-V4-A9E7	No	IIIa	565
TCGA-RZ-AB0B	No	IV	349

B, Alive patients

Submitter ID	*BAP1* mutation	Tumor stage	Days to last follow-up
TCGA-YZ-A985	Yes	IIIa	1,184
TCGA-YZ-A982	Yes	IIIb	895
TCGA-YZ-A980	Yes	IIIa	862
TCGA-WC-A880	Yes	IIIa	1,258
TCGA-VD-A8KK	Yes	IIIa	624
TCGA-VD-A8KH	No	IIIb	1,158
TCGA-VD-A8K7	No	IIIa	1,459
TCGA-V4-A9F8	No	IIIa	797
TCGA-V4-A9F5	No	IV	603
TCGA-V4-A9F4	No	IIIa	634
TCGA-V4-A9F3	No	IIIa	1543
TCGA-V4-A9F2	No	IIIa	934
TCGA-V4-A9F0	No	IIIb	1,678
TCGA-V4-A9EY	No	IIIa	837
TCGA-V4-A9EW	No	IIIa	1,211
TCGA-V4-A9EU	No	IIIc	709
TCGA-V4-A9ET	No	IIIb	1,362
TCGA-V4-A9EO	Yes	IIIa	973
TCGA-V4-A9EM	No	IIIa	966
TCGA-V4-A9EL	No	IIIa	1,082
TCGA-V4-A9EH	No	IIIb	670
TCGA-V4-A9EF	No	IIIb	1,165
TCGA-V4-A9ED	No	IIIa	1,078
TCGA-V4-A9EA	No	IIIa	1,348
TCGA-V4-A9E5	No	IIIb	1,799

TCGA, The Cancer Genome Atlas; *BAP1*, BRCA1-associated protein 1.

PRKCA serves a critical role in the growth of human melanoma cells and a low expression level of *PRKCA* promotes cellular proliferation (23). In models of melanoma, activation of *PRKCA* was associated with increased tumor cell prolifera-

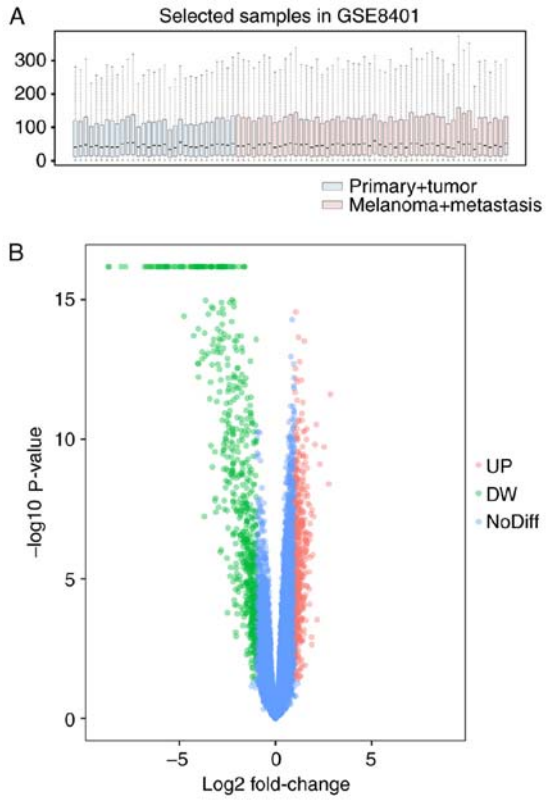

Figure 1. Data normalization and identification of differentially expressed genes in GSE8401. (A) Boxplot for normalized gene expression data and (B) volcano plot of significantly differentially expressed genes. UP, upregulated; DW, downregulated; NoDiff, no difference.

tion and invasiveness (24). Small interfering RNA-mediated knockdown of *PRKCA* resulted in a significant suppression of invasion (25). *ADCY1*, a member of the adenylate cyclase gene family, encodes adenylate cyclase. *ADCY1* demonstrated a downregulated trend in rectal adenocarcinoma metastasis (26). *LAMA1* expression was increased in esophageal squamous cell carcinoma tissues, and its overexpression rescued the multiplication suppression and cell apoptosis increases induced by microRNA-202 (27).

In the present study, *PRKCA, ADCY1* and *LAMA1* were enriched in the 'amoebiasis' signaling pathway. Colonic amoebiasis has been commonly demonstrated to mimic colon carcinoma (28-30). A rare case of colonic amoebiasis was identified to coexist with signet-ring cell carcinoma (31). All of these results support the hypothesis that PRKCA may contribute to the metastases of melanoma.

A previous study demonstrated that ADCY1 had a downregulated trend in rectal adenocarcinoma metastasis and was significantly enriched in the 'pancreatic secretion' pathway (26). In the present study, ADCY1 was identified to be involved in several melanoma-related pathways such as 'pathways in cancer' and 'melanogenesis'. Previous study has shown that melanogenesis plays a key role in regulation of cellular metabolism of melanoma cell (32).

The diagnostic gene *BUB1*, a discriminator between melanoma, benign nevi and lymph nodes, contributes to the detection of melanoma micrometastasis in sentinel lymph nodes (33). Utilizing gene microarray analysis and real-time quantitative polymerase chain reactions, the expression level of *BUB1* has been identified to be high in metastatic melanoma (34). *BUB1* is downstream of sirtuin 1 (*SIRT1*), which is upregulated in human melanoma, and its inhibition causes an anti-proliferative effect in melanoma cells (35). The expression of *BUB1* is suppressed by the inhibition of *SIRT1* (35). Therefore, high expression of *BUB1* promotes melanoma metastasis and results in a poor prognosis.

AURKA is known to induce the formation of the mitotic spindle and to be involved in cell multiplication and migration (22). *AURKA* was upregulated in malignant melanoma cells and tissues, and *AURKA* overexpression promoted human melanoma cell growth (22). Activation of *AURKA* is driven by the forkhead box protein M1 and the mitogen-activated protein kinase/extracellular-regulated kinase signaling pathway, and is associated with a poor prognosis in patients with melanoma (36). A comprehensive gene expression analysis of melanoma metastasis suggested that *BUB1* and *AURKA* may serve crucial roles in the development and progression of melanoma (37).

NDC80 is a novel oncogene that is involved in the occurrence of various tumors. It is highly expressed in a variety of cancer types, including pancreatic cancer, hepatocellular carcinoma, gastric cancer and colorectal cancer (38-41). Overexpression of *NDC80* in cancer cells and tissues promoted multiplication and migration, and was correlated with poor outcomes in those patients with pancreatic cancer (38). In patients with colorectal cancer, *NDC80* overexpression was significantly associated with advanced tumor stage and a poor prognosis (41). *NDC80* is a downstream gene of polo-like kinase 1, which is a well-documented oncogene that serves a key role in cell reproduction and invasion (42). In bladder cancer, *NDC80* was associated with the regulation of cell reproduction, migration and invasion, and was positively associated with metastasis (43). All of these results suggested that *NDC80* serves an important role in the metastases of melanoma.

PIK3R3, which activates the protein kinase B/serine/threonine-protein kinase mTOR signaling pathway, serves a crucial role in the survival, proliferation and motility of various tumors (44,45). *PIK3R3* is involved in the regulation of the actin cytoskeleton and regulates triple-negative breast cancer cell migration (46). Its expression is high in human hepatocellular carcinoma tissues and glioma tissues (45,47,48). *PIK3R3* overexpression augments tumor migration and aggressiveness, and promotes the metastasis of colorectal cancer (49). The basal expression level of *PIK3R3* was upregulated in highly metastatic human non-small-cell lung cancer cells (44).

PCNA is a proliferation marker and regulates cell growth (50). In esophageal tumor tissues, upregulation of MAGE family member D1 promoted cell growth by interacting with *PCNA* (51). *CENPM* and *UBC* were additionally identified as key candidate genes in the present study. However, *CENPM* and *UBC* have not been previously identified to be involved in cancer. Their function and roles require further experimental verification.

In addition, it was identified that the somatic mutation frequency of *BAP1* was high in patients with melanoma and patients with a low expression level of *BAP1* had poor overall survival. Conversely, high expression of *ASXL1*, *PSMD3*,

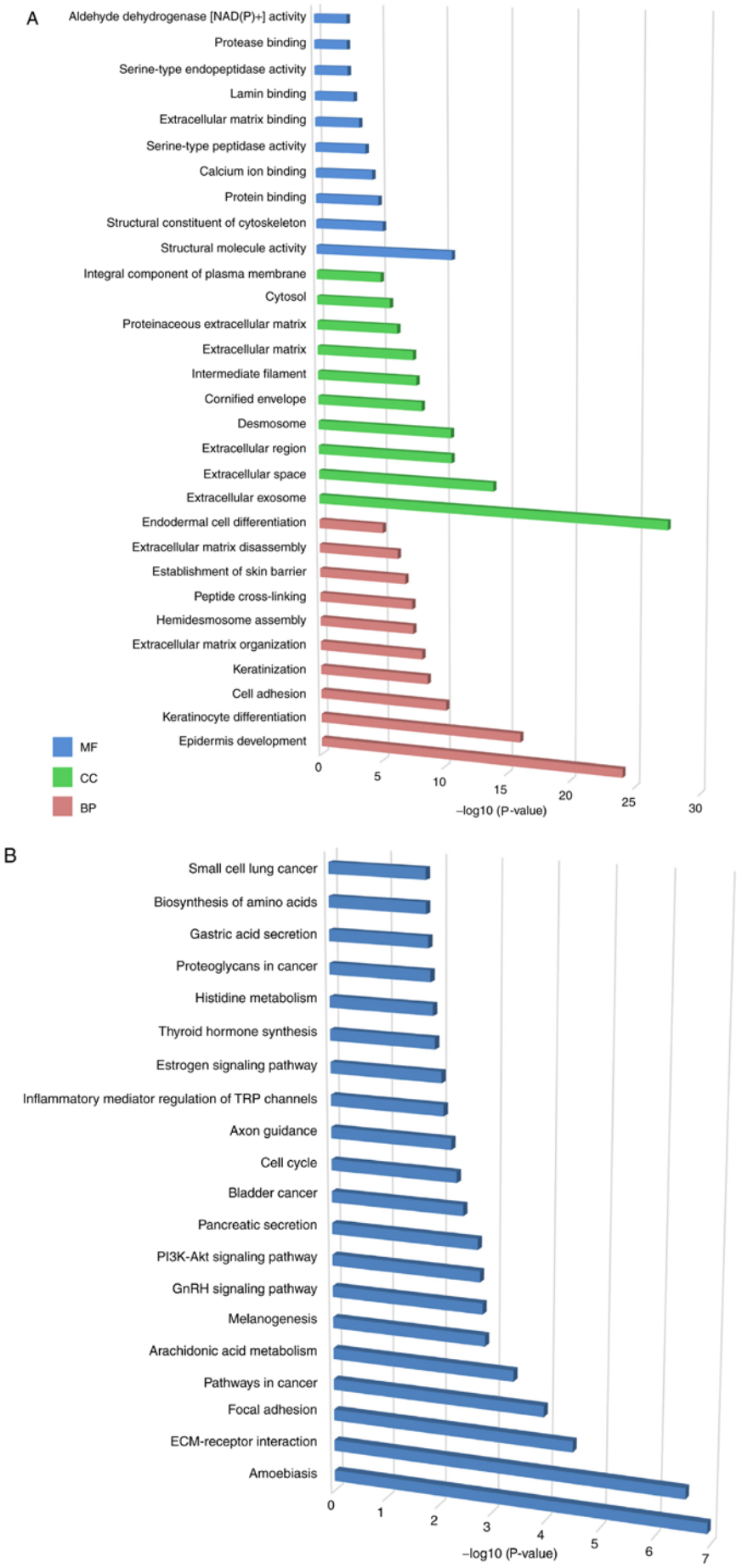

Figure 2. Gene Ontology and signaling pathway enrichment analysis. (A) Gene Ontology and (B) signaling pathway enrichment analysis of differentially expressed genes. MF, molecular function; CC, cellular component; BP, biological process.

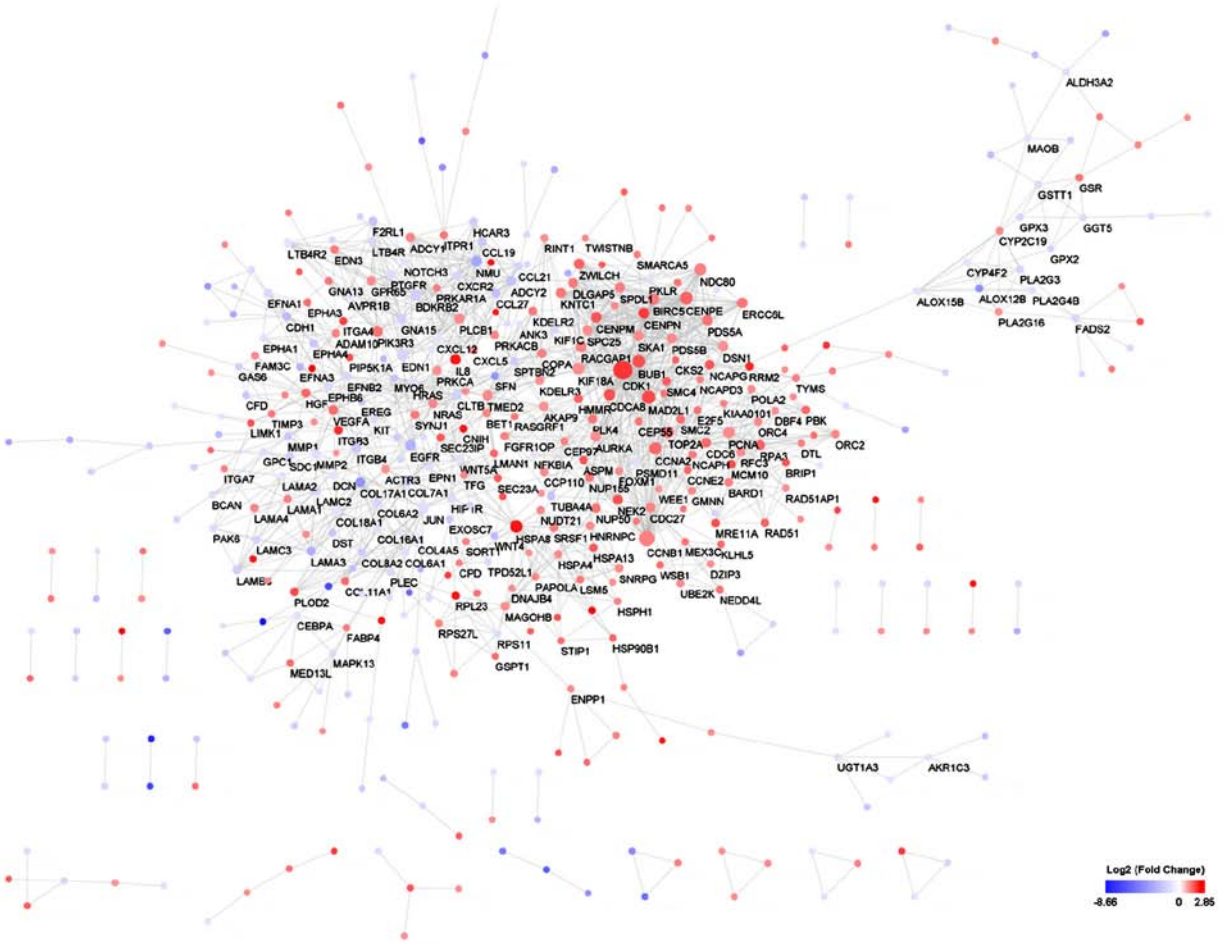

Figure 3. Differentially expressed gene protein-protein interaction network. Red nodes represent upregulated genes and blue nodes represent downregulated genes. The more the degree of the node, the bigger the nodes.

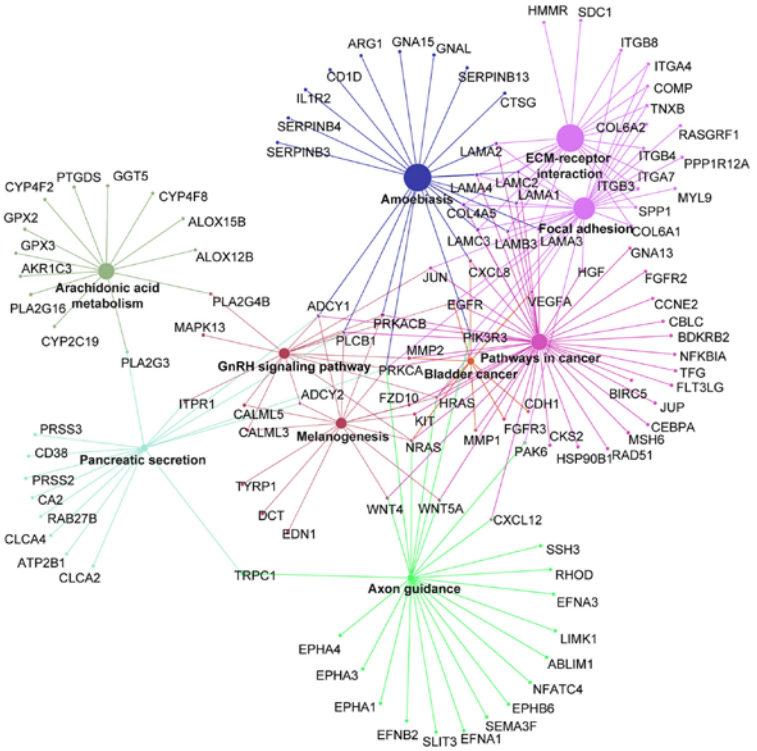

Figure 4. Differentially expressed gene Kyoto Encyclopedia of Genes and Genomes network analysis. The larger size of the nodes represent larger enrichment scores and different colors represent the different enrichment modules.

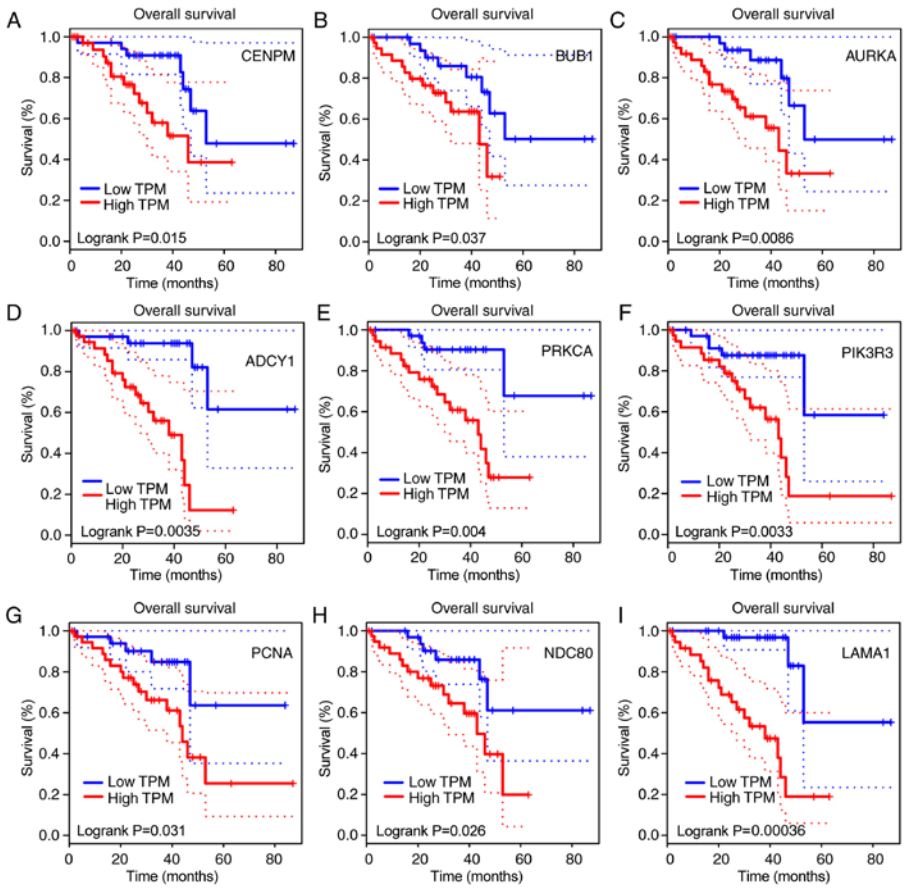

Figure 5. Overall survival curves of key candidate genes in the protein-protein network. Overall survival curves of (A) *CENPM*, (B) *BUB1*, (C) *AURKA*, (D) *ADCY1*, (E) *PRKCA*, (F) *PIK3R3*, (G) *PCNA*, (H) *NDC80* and (I) *LAMA1*. *CENPM*, centromere protein M; *BUB1*, BUB1 mitotic checkpoint serine/threonine kinase; *AURKA*, aurora kinase A; *ADCY1*, adenylate cyclase 1; *PRKCA*, protein kinase C α; *PIK3R3*, phosphoinositide-3-kinase regulator subunit 3; *PCNA*, proliferating nuclear antigen; *NDC80*, NDC80 kinetochore complex component; *LAMA1*, laminin subunit α 1; TPM, transcripts per million.

Figure 6. Top 50 mutated genes in 80 mutated cases with melanoma.

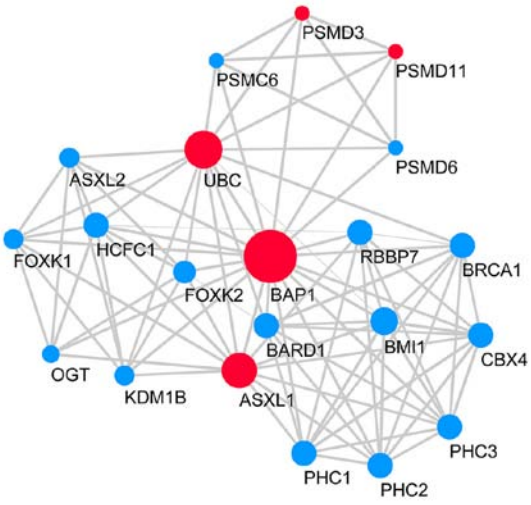

Figure 7. *BAP1*-associated gene PPI network. Red nodes represent upregulated genes and blue nodes represent downregulated genes. The larger size of the nodes represent more degrees in the global PPI network and lines indicate interactions. *BAP1*, BRCA1 associated protein 1; PPI, protein-protein interaction.

PSMD11 and *UBC*, which are *BAP1*-associated genes, was associated with poor overall survival.

BAP1 is a novel identified tumor suppressor gene and somatic mutations of *BAP1* occur in a variety of malignancies (52). *BAP1* serves a key role in stabilizing epigenetic regulators, including O-linked N-acetylglucosamine (*GlcNAc*) transferase, host cell factor C1 and *ASXL1*, and thus an ASXL/BAP1 complex may inhibit chronic myelomonocytic leukemia, in which *ASXL1* is frequently mutated (53). The frequency of *BAP1* mutations may be undervalued, and a number of previously unidentified *BAP1* and spliceosome mutations were identified in uveal melanoma (54). Inactivating mutations in *BAP1* were associated with a high metastatic risk of uveal melanoma (55). In the clinical setting, anti-BAP-1 has been used to detect the presence of BAP-1 mutation (56). Histologically, the melanocytic lesions are located predominantly in the dermis and are composed of epithelioid melanocytes with abundant cytoplasm and distinct nucleoli. Similar lesions develop in the context of somatically acquired BAP-1 loss (57). Immunohistochemistry analysis with anti-BAP-1 demonstrated loss of nuclear staining in melanocytic lesions with BAP-1 mutations (56,58). In the present study, patients with low expression levels of *BAP1* had poor overall survival, and the expression of *BAP1* was lower in metastatic melanoma compared with primary melanoma. Moreover, mutation of *BAP1* was associated with poor prognosis in 40 melanoma cases with metastasis. These results suggested that mutations of *BAP1* may serve a key role in the metastases of melanoma.

ASXL1 is involved in polycomb repressive complex 2-dependent transcriptional repression and nuclear hormone receptor- and BAP1-dependent transcriptional regulation. Somatic mutations of *ASXL* family members occur in human cancer (59). *ASXL1* overexpression was identified in cervical cancer (60) and truncation mutations in *ASXL1* were identified in various diseases, including chronic lymphocytic leukemia, malignant myeloid diseases, head and neck squamous cell carcinoma, colorectal cancer, liver cancer, prostate cancer and breast cancer (58,59). Furthermore, truncation mutations in *ASXL1* resulted in a poor prognosis of myeloid malignancies (61,62). This is inconsistent with the present result that *ASXL1* was highly expressed in metastatic melanoma and associated with poor overall survival in patients with melanoma. *ASXL1* may have different functions in the metastases of melanoma. This requires further validation.

Silencing of *PSMD3* together with human epidermal growth factor receptor 2 resulted in an additive suppression of cell viability, multiplication and protein kinase B signaling pathway activity (63). *PSMD3* is downregulated in the long-term survivor subtype of glioblastoma multiforme, suggesting that a low expression level of *PSMD3* results in longer survival (64). *PSMD11* was overexpressed in breast cancer tissue, and this may be associated with specific biological processes and pathological types of breast cancer (65). *PSMD11* is a short-lived protein, and knockdown of *PSMD11* via RNA interference partially resulted in the occurrence of acute apoptosis in pancreatic cancer cells (66). It was identified that high expression of *PSMD3* and *PSMD11* was associated with poor overall survival in patients with melanoma. Therefore, *PSMD3* and *PSMD11* may be associated with the occurrence of melanoma metastasis.

Among all of the genes discussed, the key candidate genes and the mutated genes that were identified may serve crucial roles in the metastasis of melanoma. These prognostic markers require further study, and the accuracy of diagnosis may be improved based on multiple gene detection. There are specific limitations to the present bioinformatics analysis. Only a single open GEO dataset was included in the present study, considering that there was no suitable dataset for merge analysis and verification. No functional validation was conducted to support the present findings, although a number of the present observations were supported by previous studies. Biological validation is required in future studies. The survival analysis was conducted based on a TCGA dataset with a limited number of cases. The function and role of the candidate genes in the metastases of melanoma requires further validation. Future studies may aim to validate the gene expression in clinical samples. Additionally, the diagnostic value of *BAP1* and its associated genes require validation in clinical samples. Further studies are required to investigate the molecular mechanism of the hub genes, selected in the present study, in melanoma metastasis.

In conclusion, 1,187 DEGs were identified in metastatic melanoma compared with primary melanoma. A total of 447 DEGs were mapped onto a PPI network. Based on the PPI network analysis, gene function enrichment analysis, KEGG network analysis and survival analysis, *PIK3R3*, *CENPM*, *AURKA*, *LAMA1*, *PCNA*, *ADCY1*, *BUB1*, *NDC80* and *PRKCA*, which were associated with poor overall survival, may serve key roles in the metastasis and invasion processes of melanoma. Additionally, *BAP1* and its associated genes may contribute to the metastasis and invasion processes of melanoma. The results of the present study may provide valuable prognostic markers and therapeutic targets for melanoma. Further studies are required to validate the

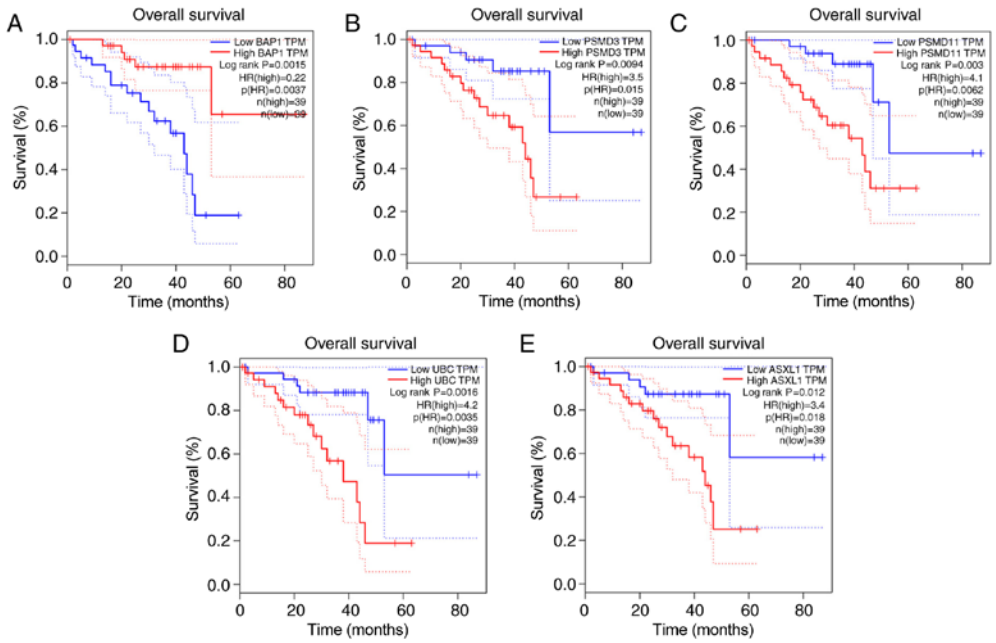

Figure 8. Overall survival curves of *BAP1* and its associated genes. Overall survival curves of (A) *BAP1*, (B) *PSMD3*, (C) *PSMD11*, (D) *UBC* and (E) *ASXL1*. *BAP1*, BRCA1 associated protein 1; *PSMD3*, proteasome 26S subunit, non-ATPase 3; *PSMD11*, proteasome 26S subunit, non ATPase 11; *UBC*, ubiquitin C; *ASXL1*, ASXL transcriptional regulator 1; TPM, transcripts per million; HR, hazard ratio.

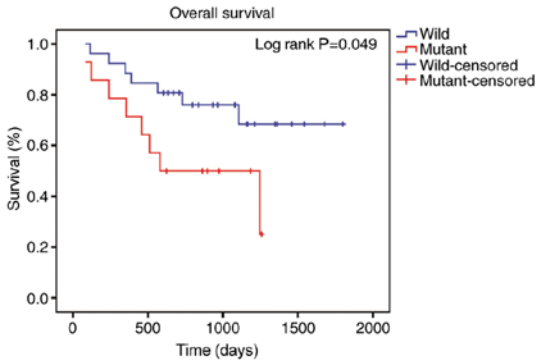

Figure 9. Overall survival curves of associated protein 1 in tumor cases with metastasis.

function and role of these DEGs in the metastasis and aggressiveness of melanoma.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JC, YLa and YLi conceived and designed the study; JC, FW and YS performed data analysis; FW designed the figures. YS and YLa reviewed the literature. JC and YLi wrote the paper. DY and MX participated in the study design and revised the paper critically for important intellectual content.

Ethics approval and consent to participate

All patient specimens were collected and used according to the approval by the institutional Ethics Committee, and written informed consent was obtained, according to the ethical standards in the 1964 Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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