Bioinformatics analysis reveals key genes and pathways that promote melanoma metastasis

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Research article

Keywords: melanoma metastasis, bioinformatical analysis, differentially expressed genes, biomarker

DOI: https://doi.org/10.21203/rs.3.rs-25246/v1

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Abstract

Melanoma has the highest mortality rate of all skin tumors, and metastases are the major cause of death from it. The molecular mechanism leading to melanoma metastasis is currently unclear. With the goal of revealing the underlying mechanism, three data sets with accession numbers GSE8401, GSE46517 and GSE7956 were downloaded from the Gene Expression Omnibus (GEO) database. After identifying the differentially expressed gene (DEG) of primary melanoma and metastatic melanoma, three kinds of analyses were performed, namely functional annotation, protein-protein interaction (PPI) network and module construction, and co-expression and drug-gene interaction prediction analysis. 41 up-regulated genes and 79 down-regulated genes were selected for subsequent analyses. Results of pathway enrichment analysis showed that extracellular matrix organization and proteoglycans in cancer are closely related to melanoma metastasis. In addition, seven pivotal genes were identified from PPI network, including CXCL8, THBS1, COL3A1, TIMP3, KIT, DCN, and IGFBP5, which have all been verified in the TCGA database, but only CXCL8, THBS1 and KIT had significant differences in expression. To conclude, CXCL8, THBS1 and KIT may be the key genes in the metastasis of melanoma and thus may be regarded as therapeutic targets in the future.

1 Background

Melanoma is the most deadly type of skin tumor and about 18% of which would show clinical metastases[1]. Each year, the epidermal spread of melanoma kills about 50000 people worldwide[2]. In recent years, it has been found that some genes are closely related to the metastasis of melanoma. A previous clinical study confirmed that NEDD9 is a bona fide melanoma metastasis gene, which enhances invasion in vitro and metastasis in vivo of both normal and transformed melanocytes. It interacts with focal adhesion kinase and modulated focal contact formation, and shows more frequent positive overexpression in metastatic melanoma than in primary melanoma[3]. In addition, studies have shown that BRAF and NRAS mutant melanomas have similar metastasis rates and they are slightly more likely to metastasize than BRAF and NRAS wild-type melanomas[4, 5]. According to other studies, up to 85% of TERT promoter mutations have been found in metastatic melanoma, while 30–40% of TERT promoter mutations have been found in primary melanoma[6]. However, the exact molecular mechanisms that promote melanoma metastasis remain less clear.

Gene chip or gene profile is a gene detection technique that has been used for more than a decade. Using gene chips can quickly detect the expression information of all the genes within the same sample time-point, which is well suited for screening differentially expressed genes[7]. However, it is difficult to achieve reliable results due to the high false positive rate of independent chip analysis. Therefore, in this study, three mRNA microarray data sets were downloaded from the Gene Expression Omnibus (GEO) for identifying differentially expressed gene (DEG) which promotes melanoma metastasis. Then, gene ontology and pathway enrichment analysis and protein-protein interaction (PPI) network analysis were performed to help us understand the molecular mechanisms of melanoma metastasis. In conclusion, a
total of 120 DEGs and 7 hub genes, which might play an important role in the metastasis of melanoma, were identified.

2 Methods

2.1 Data collection

GEO (http://www.ncbi.nlm.nih.gov/geo)[8] is a gene expression database created by NCBI, which contains high-throughput gene expression data submitted by research institutes worldwide. Three microarray datasets (GSE8401, GSE46517, and GSE7956) were downloaded from it (Affymetrix GPL96 platform, Affymetrix [HG-U133A] Affymetrix Human Genome U133A Array). The GSE8401 dataset includes 31 primary melanoma samples and 52 metastatic melanoma samples. GSE46517 consists of 31 primary melanoma samples and 73 metastatic melanoma samples. GSE7956 contains 10 poorly metastatic melanoma samples and 29 highly metastatic melanoma samples.

2.2 Identification of DEGs

The DEGs between metastatic melanoma and primary melanoma samples were screened via GEO2R (http://www.ncbi.nlm.nih.gov/geo2r). GEO2R is an interactive web tool that allows users to compare two or more datasets in a GEO series in order to identify DEGs across experimental conditions[9]. The adjusted P-values (adj. P) using Benjamini and Hochberg false discovery rate were applied to discover statistically significant genes while false-positive results corrected. Probe sets without corresponding gene symbols or genes with more than one probe set were removed or averaged, respectively. LogFC (fold change) > 0.5 and adj. P-value < 0.05 were considered statistically significant.

2.3 Enrichment analyses of DEGs

DAVID 6.8 (https://david.ncifcrf.gov/)[10] was used for enrichment analyses to investigate DEGs at the molecular and functional level. DAVID is a gene functional classification tool that integrates a set of functional annotation tools for investigators to analyze biological functions behind massive genes. In addition, the functional enrichment analysis tool (Funrich)[11] was used to analyze the biological pathways of DEGs. KOBAS 3.0 (http://kobas.cbi.pku.edu.cn)[12] was used to evaluate the pathway enrichment analyses of DEGs. Four databases, 'KEGG pathway', 'Reactome', 'BioCyc' and 'PANTHER' were for further analyses. P < 0.05 was considered significant.

2.4 Protein-protein interaction network construction

Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org) (version 10.0)[13], which is an online database of known and predicted protein interactions, was applied to predict the PPI network of DEGs. Only interactions with a combined score > 0.4 were considered statistically significant. Cytoscape (version 3.6.1, http://www.cytoscape.org) was used to visualize molecular interaction networks[14], with its CytoNCA plug-in analyzing the topological properties of nodes in the PPI network and setting parameters to no weight[15]. By ranking the scores of each node, we obtained important
nodes involved in protein interactions within the network. The Molecular Complex Detection (MCODE; version 1.5.1)[16] of Cytoscape was applied to screen the most significant module in the PPI networks with MCODE scores > 5, degree cut-off = 2, node score cut-off = 0.2, max depth = 100 and k-score = 2. The pathway enrichment analyses for the genes in the modules were conducted with KOBAS 3.0.

2.5 Hub genes selection and analyses

Considering most networks were scale-free, the hub genes were chosen with degrees ≥ 10. A network of genes and their co-expression genes was analyzed via GeneMANIA (http://www.genemania.org/)[17]. The enrichment analysis of biological processes was through Metascape (http://www.metascape.org/)[18]. The pathway enrichment analyses for the genes were conducted by KOBAS 3.0. Besides, Drug-Gene Interaction database (DGIdb) 2.0 (http://www.dgidb.org/), which mines existing resources and generates assumptions about how genes are therapeutically targeted or prioritized for drug development[19], was used in the study to predict drugs based on the module genes. The parameters were set as: preset filters: FDA approved; antineoplastic; all the default. All the drug-gene relationship pairs related to the module genes were predicted, and the network map was formed by Cytoscape. Finally, the expression of identified hub gene was verified using TCGA data which contains 102 primary melanomas and 369 metastatic melanomas.

3 Results

3.1 Identification of DEGs in metastatic melanoma

After standardizing the microarray results, DEGs (4139 in GSE8401, 2821 in GSE46517 and 350 in GSE7956) were identified. A total of 120 genes overlapped among the three datasets as shown in the Venn diagram (Fig. 1A), consisting of 79 downregulated genes and 41 upregulated genes.

3.2 Analysis of the functional characteristics of DEGs

To determine the biological functions of the above mentioned DEGs, GO enrichment analysis was performed. Results were divided into three functional categories, including biological processes (BP), cell component (CC), and molecular function (MF) (Fig. 2). For BP, DEGs were mainly enriched in cellular calcium ion homeostasis (P = 2.63 × 10^{-4}), response to wounding (P = 2.67 × 10^{-4}), cell adhesion (P = 2.88 × 10^{-4}) and biological adhesion (P = 2.92 × 10^{-4}). In terms of CC, the genes were mainly enriched in extracellular region part (P = 9.52 × 10^{-8}), extracellular region (P = 9.66 × 10^{-7}), extracellular space (P = 2.58 × 10^{-5}) and extracellular matrix (P = 6.86 × 10^{-5}). In the MF group, DEGs were significantly enriched in peptidase inhibitor activity (P = 3.8 × 10^{-3}) and calcium ion binding (P = 8.73 × 10^{-3}). A total of 120 DEGs were uploaded to Funrich, 116 of which were sent for further enrichment analysis. Funrich analysis of enriched pathways for DEGs metastasizing in melanoma showed that the DEGs were mainly enriched in the epithelial-to-mesenchymal transition (EMT), as shown in Fig. 3. According to the pathway analysis results from online database KOBAS 3.0, pathways with the top five P-values were extracellular matrix
organization \( (P = 1.11 \times 10^{-13}) \), immune system \( (P = 1.22 \times 10^{-12}) \), collagen degradation \( (P = 1.54 \times 10^{-10}) \), degradation of the extracellular matrix \( (P = 9.22 \times 10^{-10}) \) and hemostasis \( (P = 7.92 \times 10^{-9}) \) (Fig. 4A).

### 3.3 PPI network construction and module analysis

The PPI network of DEGs with combined scores greater than 0.4 was generated by Cytoscape, which contained 68 nodes and 127 interaction pairs (Fig. 1B). The most significant module \( (\text{score} = 5.429) \) from the PPI network (Fig. 1C) included 8 nodes and 19 interaction pairs. Pathway analysis results of the module genes from KOBAS 3.0 showed that pathways with the top three \( P \)-values were extracellular matrix organization \( (P = 6.51 \times 10^{-13}) \), degradation of the extracellular matrix \( (P = 5.86 \times 10^{-12}) \) and collagen degradation \( (P = 1.35 \times 10^{-10}) \) (Fig. 4B).

### 3.4 Hub gene selection and analysis

A total of 7 genes with degrees \( \geq 10 \) were identified as hub genes, namely C-X-C motif chemokine ligand 8 (CXCL8, upregulated, degree = 17), thrombospondin 1 (THBS1, upregulated, degree = 13), collagen type III alpha 1 chain (COL3A1, downregulated, degree = 12), TIMP metallopeptidase inhibitor 3 (TIMP3, upregulated, degree = 11), KIT proto-oncogene receptor tyrosine kinase (KIT, downregulated, degree = 11), decorin (DCN, downregulated, degree = 10), insulin like growth factor binding protein 5 (IGFBP5, downregulated, degree = 10). A network of the hub genes and their co-expression genes was analyzed by GeneMANIA online platform. Those 7 genes showed the complex PPI network with the Co-expression of 44.42%, Physical interactions of 40.75%, Co-localization of 13.43%, Shared protein domains of 1.31% and Predicted of 0.09% (Fig. 5B). The BP is mainly enriched in peptide cross-linking, response to mechanical stimulus and regulation of vasculature development (Fig. 5A). The pathway analyses of the hub genes were conducted using KOBAS 3.0 and pathways with the top three \( P \)-values were proteoglycans in cancer \( (P = 1.67 \times 10^{-6}) \), extracellular matrix organization \( (P = 5.2 \times 10^{-6}) \) and syndecan interactions \( (P = 5.36 \times 10^{-6}) \) (Fig. 5C). Based on the DGIdb predictions of the module genes, we obtained 32 drug-gene interaction pairs, including four upregulated genes (CXCL8, THBS1, KIT and DCN) and 30 drugs (FDA-listed + antitumor drugs), as shown in Fig. 6. Finally, the results of independence testing analysis showed that genes of CXCL8 and THBS1 had a significant increase of gene expression in metastatic melanoma, but a significant downregulation of KIT expression (Fig. 7).

### 4 Discussion

In this study, a total of 41 up-regulated genes and 79 down-regulated genes were identified from three microarray data sets and thoroughly analyzed. Pathway analyses showed that these genes are mainly involved in extracellular matrix organization and proteoglycans in cancer. Several HUB genes, CXCL8, THBS1, KIT, and DCN, were found in the PPI network, and interestingly, also found in the predicted drug-gene interactions. However, according to the independent test results of the TCGA database, the difference of CXCL8, THBS1 and KIT in expression changes was significant.
The extracellular matrix (ECM) performs many functions in addition to its structural role; as a major component of the cellular microenvironment it influences cell behaviors such as proliferation, adhesion and migration, and regulates cell differentiation and death[20]. Abnormal ECM dynamics can lead to deregulated cell proliferation and invasion, failure of cell death, and loss of cell differentiation, resulting in congenital defects and pathological processes including tissue fibrosis and cancer. Proteoglycans, as ECM constituents, is lost in aged fibroblasts, resulting in a more aligned ECM that promoted metastasis of melanoma cells[21].

CXCL8 (interleukin-8) is considered to be a typical chemokine belonging to the CXC family, responsible for the recruitment and activation of neutrophils and granulocytes at the site of inflammation. Its role in the progression of melanoma mainly depends on its interaction with specific cell surface G protein coupled receptor (GPCR), C-X-C chemokine receptor type 1 (CXCR1) and C-X-C chemokine receptor type 2 (CXCR2) [22–24]. Varney et al. examined the expression of CXCL8, its receptors, CXCR1 and CXCR2, and vessel density in human melanoma by immunohistochemical analysis of tumors from different Clark levels, depths and thicknesses, and found that the expression of CXCL8 and CXCR2 was lower in Clark level I and II specimens than in level III through V specimens and metastases[25]. It indicates that the expression of CXCL8 and CXCR2 contributes to the aggressive growth and metastasis of human malignant melanoma. Three years later, a live mouse study demonstrated that CXCR2 plays a key role in melanoma lung metastasis through a gene knockout model[26]. In addition, Wu et al. evaluated the role of CXCL8 in the growth and progression of melanoma by regulating its expression in melanoma cell lines expressing different levels of CXCL8, and found that the expression of CXCL8 is a key in regulating multiple cell phenotypes associated with melanoma growth and metastasis[27]. It shows that CXCL8 is an important biomarker in the process of melanoma metastasis.

As a matricellular glycoprotein, THBS1 regulates cellular phenotype and extracellular structure during tissue genesis and remodeling, and has been shown to regulate tumor progression and metastasis[28, 29]. There is increasing evidence that the acquisition of invasive and metastatic features of melanoma cells involves the reactivation of a developmental EMT-like program[30–32]. More importantly, the results of the biological pathway enrichment of DEGs in the study also confirmed this conclusion. As the main physiological activator of transforming growth factor-β (TGF-β), THBS1 may activate the latent TGF-β1 in the progress of melanoma to promote EMT of melanoma[33–35]. Another study also validated that increased expression of THBS1 is associated with an invasive and metastatic phenotype of melanoma, as part of a Slug-independent motility program that includes the melanoma-related VEGF/VEGFR-1 and FGF-2 pathways[36]. In addition, THBS1 has been shown to promote cell invasion of breast cancer, thyroid cancer, colon cancer and prostate cancer. Therefore, we can draw a clear conclusion that THBS1 promotes the invasion and metastasis of melanoma, which is expected to become a target for future treatment.

KIT, a tyrosine kinase receptor encoding stem cell factor, plays an important role in the development, migration and proliferation of melanocytes[37, 38]. Although KIT is expressed in some melanomas, as the disease progresses from the superficial stage to infiltration and then to the metastasis stage, the loss of
KIT expression indicates that KIT has tumor suppressive function\cite{39–41}. A recent study also found that in patients without lymph node metastasis at the initial diagnosis, the expression of KIT was significantly higher than that of patients with lymph node metastasis, indicating that melanoma with missing KIT expression is more likely to progress and metastasize\cite{42}. In addition, KIT is the target of several small molecule inhibitors such as imatinib and nilotinib. These drugs have been used clinically and can significantly extend the lifespan of patients with metastatic melanoma carrying KIT mutations\cite{43, 44}. Therefore, we believe that it mediates the metastasis of melanoma and can be used as a target for the treatment of metastatic melanoma\cite{45}.

**4 Conclusions**

In summary, the purpose of this study was to identify DEGs that may be associated with melanoma metastasis. A total of 3 key genes have been identified, which can be used as a marker for metastatic melanoma or as a drug therapy target. In addition, the differential expression of these genes has been validated in TCGA data, which made the results more reliable. However, the biological function of these genes in metastatic melanoma needs further study. We acknowledged that the study has some certain limitations. First, this is a retrospective study and all the data are from publicly available databases. Second, results require further confirmation such as by vivo or vitro experiments.

**Abbreviations**

Gene Expression Omnibus (GEO), differentially expressed gene (DEG), protein-protein interaction (PPI), Drug-Gene Interaction database (DGIdb), biological processes (BP), cell component (CC), molecular function (MF), epithelial-to-mesenchymal transition (EMT), C-X-C motif chemokine ligand 8 (CXCL8), thrombospondin 1 (THBS1), collagen type III alpha 1 chain (COL3A1), TIMP metallopeptidase inhibitor 3 (TIMP3), KIT proto-oncogene receptor tyrosine kinase (KIT), decorin (DCN), insulin like growth factor binding protein 5 (IGFBP5), extracellular matrix (ECM), G protein coupled receptor (GPCR), C-X-C chemokine receptor type 1 (CXCR1), C-X-C chemokine receptor type 2 (CXCR2), transforming growth factor-\(\beta\) (TGF-\(\beta\)).

**Declarations**

**Consent for publication**

All authors agree to publish this article.

**Ethical approval and consent to participate**

Since all materials for this study are from public databases, no ethics committee approval is required.
Author contributions statement

This article was done in collaboration with all the following authors. JQQ, JJ and YDJ determined the research theme and formulated the main research plan. SWX, GY and HB analyzed the data, explained the results, and wrote the manuscript. WJJ, WYQ and ZY helped collect data and references. All authors read and approved the final manuscript.

Competing interests

The authors declare no conflict of interests.

Fund support

None.

Availability of data and materials

All data in this article comes from an open public database and is available for free.

Acknowledgement

Thanks to all the authors who contributed to this article, and to the publisher for supporting this article.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. Cancer J Clin. 2015;65(2):87–108.
2. Brehmer F, Ulrich M, Haenssle HA. Strategies for early recognition of cutaneous melanoma-present and future. Dermatology practical conceptual. 2012;2(3):203a206.
3. Kim M, Gans JD, Nogueira C, Wang A, Paik JH, Feng B, Brennan C, Hahn WC, Cordon-Cardo C, Wagner SN, et al. Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. Cell. 2006;125(7):1269–81.
4. Colombino M, Capone M, Lissia A, Cossu A, Rubino C, De Giorgi V, Massi D, Fonsatti E, Staibano S, Nappi O, et al. BRAF/NRAS mutation frequencies among primary tumors and metastases in patients with melanoma. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2012;30(20):2522–9.
5. Jakob JA, Basset RL Jr, Ng CS, Curry JL, Joseph RW, Alvarado GC, Rohlfs ML, Richard J, Gershenwald JE, Kim KB, et al. NRAS mutation status is an independent prognostic factor in
metastatic melanoma. Cancer. 2012;118(16):4014–23.

6. Huang FW, Hodis E, Xu MJ, Kryukov GV, Chin L, Garraway LA. Highly recurrent TERT promoter mutations in human melanoma. Science. 2013;339(6122):957–9.

7. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013;339(6127):1546–58.

8. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic acids research. 2002;30(1):207–10.

9. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, et al. NCBI GEO: archive for functional genomics data sets–update. Nucleic acids research. 2013;41(Database issue):D991–5.

10. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nature protocols. 2009;4(1):44–57.

11. Pathan M, Keerthikumar S, Ang C-S, Gangoda L, Quek CYJ, Williamson NA, Mouradov D, Sieber OM, Simpson RJ, Salim A, et al. FunRich: An open access standalone functional enrichment and interaction network analysis tool. Proteomics. 2015;15(15):2597–601.

12. Wu J, Mao X, Cai T, Luo J, Wei L. KOBASE server: a web-based platform for automated annotation and pathway identification. Nucleic acids research. 2006;34:W720–4. (Web Server issue).

13. Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, Lin J, Mingez P, Bork P, von Mering C, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic acids research. 2013;41(Database issue):D808–15.

14. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics. 2011;27(3):431–2.

15. Tang Y, Li M, Wang J, Pan Y, Wu F-X. CytoNCA: a cytoscape plugin for centrality analysis and evaluation of protein interaction networks. Biosystems. 2015;127:67–72.

16. Saito R, Smoot ME, Ono K, Ruscheinski J, Wang P-L, Lotia S, Pico AR, Bader GD, Ideker T. A travel guide to Cytoscape plugins. Nat Methods. 2012;9(11):1069–76.

17. Warde-Farley D, Donaldson SL, Comes O, Zuberi K, Badrawi R, Chao P, Franz M, Grouios C, Kazi F, Lopes CT, et al: The GeneMANIA prediction server: biological network integration for gene prioritization and predicting gene function. Nucleic acids research 2010, 38(Web Server issue):W214-220.

18. Zhou Y, Zhou B, Pache L, Chang M, Khodabakhshi AH, Tanaseichuk O, Benner C, Chanda SK: Metascape provides a biologist-oriented resource for the analysis of systems-level datasets. In: Nature communications. vol. 10; 2019: 1523.

19. Wagner AH, Coffman AC, Ainscough BJ, Spies NC, Skidmore ZL, Campbell KM, Krysiak K, Pan D, McMichael JF, Eldred JM, et al: DGIdb 2.0: mining clinically relevant drug-gene interactions. Nucleic acids research 2016, 44(D1):D1036-1044.

20. Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326(5957):1216–9.
21. Kaur A, Ecker BL, Douglass SM, Kugel CH 3rd, Webster MR, Almeida FV, Somasundaram R, Hayden J, Ban E, Ahmadzadeh H, et al. Remodeling of the Collagen Matrix in Aging Skin Promotes Melanoma Metastasis and Affects Immune Cell Motility. Cancer discovery. 2019;9(1):64–81.

22. Waugh DJ, Wilson C. The interleukin-8 pathway in cancer. Clinical cancer research: an official journal of the American Association for Cancer Research. 2008;14(21):6735–41.

23. Singh S, Nannuru KC, Sadanandam A, Varney ML, Singh RK. CXCR1 and CXCR2 enhances human melanoma tumourigenesis, growth and invasion. British journal of cancer. 2009;100(10):1638–46.

24. Singh S, Sadanandam A, Varney ML, Nannuru KC, Singh RK. Small interfering RNA-mediated CXCR1 or CXCR2 knock-down inhibits melanoma tumor growth and invasion. International journal of cancer. 2010;126(2):328–36.

25. Varney ML, Johansson SL, Singh RK. Distinct expression of CXCL8 and its receptors CXCR1 and CXCR2 and their association with vessel density and aggressiveness in malignant melanoma. Am J Clin Pathol. 2006;125(2):209–16.

26. Singh S, Varney M, Singh RK. Host CXCR2-dependent regulation of melanoma growth, angiogenesis, and experimental lung metastasis. Cancer research. 2009;69(2):411–5.

27. Wu S, Singh S, Varney ML, Kindle S, Singh RK. Modulation of CXCL-8 expression in human melanoma cells regulates tumor growth, angiogenesis, invasion, and metastasis. Cancer Med. 2012;1(3):306–17.

28. Carlson CB, Lawler J, Mosher DF. Structures of thrombospondins. Cell Mol Life Sci. 2008;65(5):672–86.

29. Miyata Y, Sakai H. Thrombospondin-1 in urological cancer: pathological role, clinical significance, and therapeutic prospects. Int J Mol Sci. 2013;14(6):12249–72.

30. Wehbe M, Soudja SM, Mas A, Chasson L, Guinamard R, de Tenbossche CP, Verdeil G, Van den Eynde B, Schmitt-Verhulst AM. Epithelial-mesenchymal-transition-like and TGFbeta pathways associated with autochthonous inflammatory melanoma development in mice. PloS one. 2012;7(11):e49419.

31. Caramel J, Papadogeorgakis E, Hill L, Browne GJ, Richard G, Wierinckx A, Saldanha G, Osborne J, Hutchinson P, Tse G, et al. A switch in the expression of embryonic EMT-inducers drives the development of malignant melanoma. Cancer cell. 2013;24(4):466–80.

32. Kim JE, Leung E, Baguley BC, Finlay GJ. Heterogeneity of expression of epithelial-mesenchymal transition markers in melanocytes and melanoma cell lines. Frontiers in genetics. 2013;4:97.

33. Bige N, Shweke N, Bennassine S, Jouanneau C, Vandermeersch S, Dussaule JC, Chatziantoniou C, Ronco P, Boffa JJ. Thrombospondin-1 plays a profibrotic and pro-inflammatory role during ureteric obstruction. Kidney international. 2012;81(12):1226–38.

34. Xie F, Ling L, van Dam H, Zhou F, Zhang L. TGF-beta signaling in cancer metastasis. Acta Biochim Biophys Sin. 2018;50(1):121–32.

35. Jayachandran A, Anaka M, Prithviraj P, Hudson C, McKeown SJ, Lo PH, Vella LJ, Goding CR, Cebon J, Behren A. Thrombospondin 1 promotes an aggressive phenotype through epithelial-to-mesenchymal transition in human melanoma. Oncotarget. 2014;5(14):5782–97.
36. Borsotti P, Ghilardi C, Ostano P, Silini A, Dossi R, Pinessi D, Foglieni C, Scatolini M, Lacal PM, Ferrari R, et al. Thrombospondin-1 is part of a Slug-independent motility and metastatic program in cutaneous melanoma, in association with VEGFR-1 and FGF-2. Pigment cell melanoma research. 2015;28(1):73–81.

37. Grichnik JM, Burch JA, Burchette J, Shea CR. The SCF/KIT pathway plays a critical role in the control of normal human melanocyte homeostasis. J Invest Dermatol. 1998;111(2):233–8.

38. Alexeev V, Yoon K. Distinctive role of the cKit receptor tyrosine kinase signaling in mammalian melanocytes. J Invest Dermatol. 2006;126(5):1102–10.

39. Huang S, Luca M, Gutman M, McConkey DJ, Langley KE, Lyman SD, Bar-Eli M. Enforced c-KIT expression renders highly metastatic human melanoma cells susceptible to stem cell factor-induced apoptosis and inhibits their tumorigenic and metastatic potential. Oncogene. 1996;13(11):2339–47.

40. Montone KT, van Belle P, Elenitsas R, Elder DE. Proto-oncogene c-kit expression in malignant melanoma: protein loss with tumor progression. Mod Pathol. 1997;10(9):939–44.

41. Natali PG, Nicotra MR, Winkler AB, Cavaliere R, Bigotti A, Ullrich A. Progression of human cutaneous melanoma is associated with loss of expression of c-kit proto-oncogene receptor. International journal of cancer. 1992;52(2):197–201.

42. Dai B, Cai X, Kong Y-Y, Yang F, Shen X-X, Wang L-W, Kong J-C. Analysis of KIT expression and gene mutation in human acral melanoma: with a comparison between primary tumors and corresponding metastases/recurrences. Hum Pathol 2013, 44(8):1472–1478.

43. Kong Y, Si L, Zhu Y, Xu X, Corless CL, Flaherty KT, Li L, Li H, Sheng X, Cui C, et al. Large-scale analysis of KIT aberrations in Chinese patients with melanoma. Clinical cancer research: an official journal of the American Association for Cancer Research. 2011;17(7):1684–91.

44. Guo J, Carvajal RD, Dummer R, Hauschild A, Daud A, Bastian BC, Markovic SN, Queirolo P, Arance A, Berking C, et al. Efficacy and safety of nilotinib in patients with KIT-mutated metastatic or inoperable melanoma: final results from the global, single-arm, phase II TEAM trial. Ann Oncol. 2017;28(6):1380–7.

45. Carvajal RD, Antonescu CR, Wolchok JD, Chapman PB, Roman R-A, Teitcher J, Panageas KS, Busam KJ, Chmielowski B, Lutzky J, et al. KIT as a therapeutic target in metastatic melanoma. JAMA. 2011;305(22):2327–34.

Figures
Venn diagram, PPI network and the most significant module of DEGs. (A) DEGs were selected with a fold change >0.5 and P-value <0.05 among the mRNA expression profiling sets GSE8401, GSE46517 and GSE7956. The 3 datasets showed an overlap of 120 genes. (B) The PPI network of DEGs was constructed using Cytoscape. (C) The most significant module was obtained from PPI network with 8 nodes and 19 edges. Upregulated genes are marked in light red; downregulated genes are marked in light blue.

Figure 1
Gene Ontology analyses of differentially expressed genes (DEGs) between primary melanomas and metastatic melanomas. The biological process in functional enrichment of DEGs was performed using the online biological tool DAVID between primary melanomas and metastatic melanomas with P value (A) and gene count (B).
Figure 3

The biological pathways of DEGs by Funrich.
Figure 4

The pathway analysis of DEGs by KOBAS 3.0. (A) The pathway analysis of all the DEGs. (B) The pathway analysis of the genes in the most important modules.
Figure 5

Interaction network, biological process and pathway analysis of the hub genes. (A) The top 5 enriched GO categories of biological process via Metascape. (B) Hub genes and their co-expression genes were analyzed using GeneMANIA. (C) The pathway analysis of the hub genes by KOBAS 3.0.
Figure 6

Drug-gene interaction diagram, yellow circle indicates the differentially expressed gene and blank square indicates the drug.
Figure 7

Hub genes expression in the TCGA. P<0.05 was considered statistically significant.