Identification of biomarkers and pathways associated with immune infiltration and prognosis in melanoma patients

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Abstract

Background Skin cutaneous melanoma (SKCM) is one of the most malignancy and aggressive cancers, causing about 72% of deaths in skin carcinoma. Although extensive study has explored the mechanism of recurrence and metastasis, the tumorigenesis of melanoma remains unclear. Exploring the tumorigenesis mechanism may help to identify prognostic biomarkers that could serve to guide cancer therapy .

Methods We identified differentially expressed genes (DEGs) between patients with primary melanoma and normal skin in three data sets including GSE46517, GSE15605, GSE114445. Functional annotation including KEGG pathway and gene ontology (GO) enrichment analysis was performed by DAVID. Subsequently, protein - protein interaction network of DEGs was developed and the most significant module was constructed as hub genes for further study. ClueGO was used to investigate significant pathways in hub genes. The Kaplan–Meier method was performed to assess prognostic genes. ROC curves were used to describe diagnosis value of hub genes. Then, genes expression and clinical characteristics were validated by TCGA profiles. In addition, we explored the correlation between prognostic genes and immune cell infiltration.

Results A total of 308 DEGs and 12 hub genes were identified. GO enrichment results demonstrated a correlation between DEGs and the immune response, inflammatory response and transcriptional control. Hallmark pathways of hub genes including interleukin-10 signaling, chemokine receptors bind chemokine signaling & peptide ligand-binding receptors. Survival analysis and ROC curves suggested that CCL4, CCL5, NMU, CXCL9, CXCL10, CXCL13 were independent prognostic factors (except GAL). Furthermore, prognostic genes were highly expressed in tumor tissue and related to pathological stages and Breslow depth. In addition, the expressions of CCL4, CCL5, CXCL9, CXCL10, CXCL13 were positively correlated with six immune cell infiltration (B-cell, CD8+ T cells, CD4+ T cells, macrophages, Neutrophils, Dendritic cells) and 28 types of TILs such as activated CD8 T cells, regulatory T cells, natural killer T cells while NMU and GAL were negatively correlated with it.

Conclusion In summary, this study identified significant hub genes and pathways closely associated with immune infiltrations and prognosis in SKCM patients, which might help us evaluate underlying carcinogenesis progress from skin to melanoma.

Background

Skin cutaneous melanoma (SKCM) accounts for only 2% of total skin cancer. However, due to its high degree of malignancy and invasiveness, it causes about 72% of deaths in skin carcinoma [1]. The incidence of cutaneous malignant melanoma continue to increase annually [2]. Melanoma has become a serious public health problem, bringing great economic burden for society [3]. It is well known that melanoma is associated with multiple risk factors especially the sun exposure [4]. The general progression models of melanoma are from melanocyte to melanoma in situ, to invasive melanoma [5].

However, extensive research has explored the mechanism of recurrence and metastasis, the tumorigenesis of melanoma remains unclear. Cancer cells act as antigens to induce immune infiltration while immune cells are attracted to tumor environment through chemotaxis to play immune defense roles [6]. In addition, increasing researches showed that immune escape is an important factor in tumorigenesis [7]. Emerging immunotherapy strategies have been applied in melanoma such as anti-PD-1, anti-PDL-1, anti-CTLA4 and MAGE-A3 [8]. However, only a small group of patients benefit from it and 50%-60% showed poor prognosis [9]. The tumor microenvironment (TME) consists of a variety of immune cells and stromal cells, including immune cells, fibroblasts, endothelial cells, extracellular matrix, cytokines, chemokines and receptors [10]. Previous researches have suggested that the increasing of tumor-infiltrating lymphocytes (TILs) was associated with better prognosis, reduced lymph node metastasis and a lengthier disease-free survival [11]. Therefore, exploration of the mechanism of melanoma and identification the immune-related biomarkers with better prognosis will greatly help us evaluate underlying carcinogenesis progress of melanoma, which might improve the precision medicine and immunotherapies.

In the present study, we analyzed gene expression profiles to identify hub genes and pathways associated with immune infiltrations and prognosis. Our results may reveal potential therapeutic targets and provide insights into the molecular mechanisms of melanoma.

Methods

Gene expression datasets

Expression profiling of melanoma patients with clinical information was obtained from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo>) [12]. Among the inclusion criteria were (a) diagnosis of patients with primary melanoma (PM) and normal skin (NS), (b) detection of gene level in tissue or blood samples. Exclusion criteria included: (a) clinical data without survival time and outcome, and (b) datasets with small sample sizes ($n < 16$). Finally, three datasets were eligible: accession numbers GSE15605 (46 PM samples and 16 NS samples), GSE46517 (31 PM samples and 8 NS samples) and GSE114445 (16 PM samples and 6 NS samples). For validation, level 3 data of gene expression profiles of 472 melanoma patients were downloaded from the TCGA data portal (<https://tcga-data.nci.nih.gov/tcga/>), measured experimentally using the Illumina High-Sequence. Clinical characteristics of patients were also obtained from the TCGA data portal, including age, Clark level, Breslow depth value, survival time, and outcome. Clinicopathological characteristics of SKCM patients from GEO and TCGA were shown in Table1.

Identification of DEGs

The DEGs between primary melanoma and normal skin were identified using GEO2R (<http://www.ncbi.nlm.nih.gov/geo/geo2r>) based on limma package with the threshold of $|\log FC| > 1$ and P value < 0.05 which were considered of statistically significance. For the next step, the online Venn

software (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was applied to detect the overlap DEGs among three datasets.

Function enrichment analysis for DEGs

To elucidate the biological functions of the overlapping DEGs, we performed functional annotation and pathway enrichment analysis via DAVID (The Database for Annotation, Visualization and Integrated Discovery, <http://david.ncifcrf.gov/>, version 6.8) online tool [13]. P value <0.05 was considered statistically significant. GO enrichment and KEGG pathway of DEGs was analyzed and displayed using bubble charts.

PPI network construction and hub genes selection

STRING (Search Tool for the Retrieval of Interacting Genes, <http://string-db.org>, version 11.0) online database was used to predict the PPI network of DEGs and analyze the functional interactions between proteins [14]. An interaction with a combined score > 0.4 was recognized as statistical significance. The molecular interaction networks were visualized using the Cytoscape (version 3.4.0) and the most significant hub genes in the PPI network was narrowed down using MCODE (Cytoscape) with the following criteria: degree cutoff = 2, node score cutoff = 0.2, k-core = 2, max depth = 100 [15,16].

Hub genes significant pathways analysis

The pathway analysis of hub genes was performed and visualized by ClueGO (version 2.5.4) and CluePedia (version 1.5.4), which was a functional extension of ClueGO and a plug-in of Cytoscape [17]. p-value < 0.01 was considered statistically significant.

Prognostic and diagnostic value assessment of hub genes

The Kaplan-Meier method was used to analyze survival differences between groups based on TCGA cohort. The primary endpoint was Overall survival (OS). The follow-up duration was estimated and illustrated by the Kaplan-Meier method with 95% confidence intervals (95%CI) and log-rank test in separating curves. ROC curves were constructed to assess the diagnostic accuracy of the prognostic genes and statistical analysis was performed using SPSS 22.0. A value of P<0.01 was considered statistically significant.

Validation of prognostic genes expression and clinical characteristics

A total of 472 melanoma patients with available RNA sequence data from TCGA database and GEPIA (<http://gepia.cancer-pku.cn/index.html>) database were used to verify gene expression and clinical characteristics [18]. The overall statistical expression difference of pathological stages or Breslow depth was measured using One-way ANOVA test. Student's t tests were used to compare differential transcriptional expressions levels of prognostic genes between paired tumor and normal samples. P-values less than 0.05 were considered significant. The Human Pathology Atlas project (<https://www.proteinatlas.org>) contains immunohistochemistry (IHC) data using a tissue microarray-

based analysis on 44 different normal tissue types, and proteome analysis of 17 major cancer types [19]. In this study, representative proteins expressions of IHC images of prognostic genes were detected in melanoma and normal tissues in Human Protein Atlas.

Immune infiltration analysis

Prognostic gene expression data were used to measure the abundance of six types of infiltrating immune cells (B cells, CD4+T cells, CD8+T cells, neutrophils, macrophages, and dendritic cells) in melanoma patients using the Tumor Immune Estimation Resource (TIMER) algorithm [20]. An integrated repository portal for tumor-immune system interactions (TISIDB, <http://cis.hku.hk/TISIDB/index.php>) was utilized to examine tumor and immune system interactions in 28 types of TILs across human cancers [21]. Spearman's test was used to measure correlations between prognostic genes and TILs. All hypothetical tests were two-sided and p-values <0.05 were considered significant in all tests.

Drug–gene interaction predictive analysis

The Drug–Gene Interaction database (DGIdb2.0) mines existing resources and generates assumptions about how genes are therapeutically targeted or prioritized for drug development [22]. In this study, DGIdb2.0 was used to predict drugs based on the module hub genes. The parameters were set as: preset filters: FDA approved; antineoplastic; all the default. All the drug–gene relationship pairs related to the hub genes were predicted.

Results

Identification of DEGs

After standardization and identification of the microarray results, DEGs (4640 in GSE15065, 1096 in GSE46517 and 1895 in GSE114445) were selected. The overlap among three datasets included 308 genes as shown in the Venn diagram (Figure 2a) (Additional file 1) between primary melanoma and normal skin.

GO and KEGG Enrichment Analysis of the DEGs

Functional and pathway enrichment of DEGs was performed by DAVID, displayed in bubble charts ($p < 0.05$, Figure 3). GO analysis indicated that changes in biologic processes of DEGs were significantly enriched in the inflammatory response, immune response, regulation of transcription. In addition to cellular component, the overlapping DEGs were particularly enriched in extracellular region, extracellular space and extracellular exosome. Changes in molecular function were mostly enriched in chemokine activity, transcriptional activator activity, protein binding and CXCR3 chemokine receptor binding. At last, KEGG pathway analysis demonstrated that DEGs played pivotal roles in pathways in cytokine-cytokine receptor interaction, chemokine signaling pathway and pathways in cancer. These results demonstrated a correlation between DEGs and immune response and inflammatory response.

PPI network construction and hub genes analysis

The PPI network was constructed via Cytoscape, including 247 nodes and 792 edges (Figure 2b). Then, the most important PPI network module was obtained using MCODE, consisted of 12 nodes and 64 edges (Figure 2c). After selection, CCL5, PTGER3, IL6, CXCL13, CCL27, CCL4, CXCL9, NMU, CXCL2, GAL, NPY1R, CXCL10 were considered as hub genes of the network. The pathway analysis of hub genes via ClueGO (Figure 4) revealed that the hallmark pathways include interleukin-10 signaling, peptide ligand-binding receptors and chemokine receptors bind chemokine signaling.

Prognostic and diagnostic value assessment of hub genes

We found that CCL4, CCL5, CXCL9, CXCL10, CXCL13 were significantly positively associated with overall survival (OS) and NMU, GAL were negatively related to OS ($P < 0.01$) (Figure 5). In addition, CCL4 (AUC=0.872), CCL5 (AUC=0.775), CXCL9 (AUC=0.834), CXCL10 (AUC=0.786), CXCL13 (AUC=0.807), NMU (AUC=0.829) except GAL (AUC=0.536) were of diagnostic value with p value < 0.001 (Figure 6).

Validation of prognostic genes expression and clinical characteristics

In the present study, 472 melanoma patients from the TCGA cohort were used to analyze correlation between prognostic gene expression and pathological stages, Breslow depth, which are useful prognostic indicators for melanoma. As shown in figure 7, CXCL9, 10, 13 and CCL4, 5 were significantly overexpressed in stage I and decreased in subsequent stages with P value < 0.001 . What's more, the expressions of CXCL9, 10 and CCL4 ($p < 0.05$) were negatively correlated with Breslow depth from (0-1.5mm) to (> 3 mm) while NMU, GAL ($P < 0.05$) were positively correlated with it (Figure 8). Moreover, CXCL9, 10, 13 and CCL4, 5, NMU were high expressed in tumor tissue compared to normal ($p < 0.01$, Figure 9). IHC images suggested that CCL4, 5, CXCL9, 10, 13 and NMU were detected in melanoma tissues while not detected in normal tissues (Figure 10). All the results suggested these hub genes are of important prognostic value.

Immune infiltration analysis

After determining the prognostic value, we performed correlation analysis between prognostic genes and immune infiltration level for melanoma. There was a positive correlation between CXCL9 expression and the infiltration of B cells (Cor=0.238, $p = 3.58e-07$), CD8+T cells (Cor=0.643, $p = 1.94e-52$), CD4+T cells (Cor=0.299, $p = 1.19e-10$), macrophages (Cor = 0.247, $p = 9.59e-8$), neutrophils (Cor = 0.612, $p = 7.03e-48$), and dendritic cells (Cor = 0.648, $p = 1.56e-54$; Figure 11a). Similar results were obtained for CXCL10, CXCL13, CCL4 and CCL5 (Figure 11b-e). While GAL was found negatively correlated with infiltration of CD8+T cells (Cor= -0.171, $p = 3.22e-4$), CD4+T cells (Cor= -0.108, $p = 2.30e-2$), neutrophils (Cor = -0.185, $p = 7.94e-5$), and dendritic cells (Cor = -0.135, $p = 4.42e-3$; Figure 11g). Additionally, we also found significant correlations of these seven genes with 28 types of TILs across human heterogeneous cancers (Figure 12). CXCL9 significantly positive correlated with abundance of 28 types TILs such as activated CD8 T cells (Act_CD8 T cells; $\rho = 0.801$, $p < 2.2e-16$), regulatory T cells (Treg; ρ

= 0.693, $p < 2.2e-16$), macrophage ($\rho = 0.655$, $p < 2.2e-16$), natural killer T cells (NK T cells; $\rho = 0.74$, $p < 2.2e-16$), myeloid derived suppressor cells (MDSC; $\rho = 0.744$, $p < 2.2e-16$), immature B cell (Imm_B; $\rho = 0.78$, $p < 2.2e-16$) in Figure 12a. Similar results were found in CXCL10, CXCL13, CCL4 and CCL5 (Figure 12b-e). While NMU was negatively correlated with abundance of TILs (Act_CD8 T cells; $\rho = -0.159$, $p < 0.001$, Treg; $\rho = -0.138$, $p < 0.01$, macrophage; $\rho = -0.108$, $p < 0.05$, NK T cells; $\rho = -0.176$, $p < 0.001$, MDSC; $\rho = -0.094$, $p < 0.05$, Imm_B; $\rho = -0.207$, $p < 0.001$) and similar results were found in GAL as shown in Figure 12f-g.

Drug–gene interaction prediction

We obtained 42 drug-gene interaction pairs in DGIdb, including genes (IL6, CXCL2, CXCL10, CCL4, CCL5, PTGER3, GAL, NPY1R) and 69 drugs, as shown in Figure 13. This result may help to develop new targets for melanoma therapy.

Discussion

Skin is the largest organ of human beings. There are about 1,500 melanocytes in human epidermis per square millimeter, equivalent to nearly 3 billion melanocytes in an ordinary human skin [23]. The incidence of melanoma continues to increase every year. Once melanoma spreads through the dermis, the prognosis is poor and melanoma is not projected to reduce the death rate in the next few years [24]. A series of therapeutic methods, such as radiotherapies, chemotherapies, targeted therapies and immunotherapies have enhanced advanced patient survival [25]. However, many patients that are being administered these therapies demonstrate a low durable response, drug resistance and poor prognosis [26]. It is imperative to mine the mechanism in melanoma and to detect effective prognostic biomarkers that could serve to guide cancer therapy.

In the present study, 308 DEGs were identified and related to inflammatory response, immune response and regulation of transcription. Moreover, the PPI network were constructed and nodes with a high connectivity degree in the modules were consider as hub genes. Hallmark pathways in hub genes include interleukin-10 signaling, chemokine receptors bind chemokine signaling and peptide ligand-binding receptors. STAT3 is essential for the action of IL-10,IL-6, and IL-6 plays a central role in melanoma development and enhances the IL-10 production via STAT3-dependent signaling [27]. IL-6,10/JAK-STAT3 pathways are found in many carcinomas, and their hyperactivation was generally related to unfavorable clinical prognosis [28]. IL-6, IL-10 create a favorable environment to support tumorigenesis by increasing the cancer cell proliferation, angiogenesis, metastasis and contribute to enhance the immune suppression [28]. Logan K. Smith et al. found that interleukin-10 directly inhibits CD8+T cell function by enhancing N-Glycan branching to decrease antigen sensitivity [29]. However, chemokine and its receptors usually attract immune cells such as CD8+T cell, NKs in tumor microenvironment for immune activation [30]. Apparently, these two pathways play opposite roles in tumor formation, and the tumorigenesis of melanoma may be related to their abnormality. Kaplan-Meier method suggested that CCL4, CCL5, NMU, GAL, CXCL9, CXCL10, CXCL13 were significant prognostic signatures for SKCM. The prognostic genes

were verified to be differently expressed in tumor tissues and associated with pathological stages, Breslow depth, which indicated their important prognostic value. In addition, the expressions of CCL4, CCL5, CXCL9, CXCL10, CXCL13 were positively correlated with six immune cell infiltration (B-cell, CD8+ T cells, CD4+ T cells, macrophages, Neutrophils, Dendritic cells) and 28 types of TILs such as activated CD8 T cells, regulatory T cells, natural killer T cells and myeloid derived suppressor cells. While NMU and GAL were negatively correlated with infiltration of immune cells and TILs. Previous studies suggest that high levels of immune cell infiltration are associated with favorable outcomes [31]. A novel study based on thousands of melanoma patients found that the degree of lymphocyte infiltration was an independent prognosticator of disease-free survival (DFS), such that a lesser level was associated with a reduced DFS [32]. We predicted drug-gene interaction pairs between hub genes and 69 drugs. Our results may reveal immune-related targets for cancer therapy.

C-X-C motif ligand 9,10 (CXCL9, CXCL10) belong to CXC family chemokine. They are predominantly expressed by immune cells such as macrophages and T cells. CXCL9, CXCL10 exert their function through binding to C-X-C motif receptor CXCR3 that was highly expressed on the activated T cells [30]. Research shows that CD8+ T cell infiltration is CXCR3 dependent [33]. CD8+ T cell plays important role in tumor microenvironment, which inhibits the proliferation and metastasis of tumor cells. In our study, high expression of CXCL9 and CXCL10 associated with better overall survival in melanoma patients. What's more, CXCL9 and CXCL10 were found to be highly expressed in stage I melanoma compared to other stages. A recent research found that the CXCL10-CXCR3 axis may relate to melanoma brain metastasis [34]. Additionally, the CXCR3 ligands enhanced the response rates to immune checkpoint blockade (anti-PD-1, anti-CTLA-4) by recruiting T cells [35]. Kim J et al. found that CXCR3-deficient natural killer cells fail to migrate to B16F10 melanoma cells [36]. These results reveal the importance of manipulating this axis, which provide us opportunities to manipulate chemokine expression for tumor therapy and prevention.

C-X-C motif ligand 13 (CXCL13) also belongs to CXC family. There are very few studies on the relationship between CXCL13 and melanoma. One research suggested that CXCL13 is closely interact with NRAS, BAF and MITF in MAPK signaling pathway, which have implications for melanoma genesis and metastatic progression [37]. In our study, CXCL13 showed similar outcome in overall survival and expression with CXCL9, CXCL10. Pathway analysis suggested they were involved in peptide ligand-binding receptors and chemokine receptors bind chemokine. Therefore, CXCL13 may play the same role as CXCL9, CXCL10 in the occurrence and development of melanoma. Our results revealed that CXCL9, CXCL10, CXCL13 are positively associated with immune cell infiltration. Interestingly, CXCL10 and CXCL2 were found in the predicted drug-gene interaction, providing new thoughts for melanoma treatment. Whether to detect the level of chemokine expression in peripheral blood can help us diagnose melanoma and its stage is unknown. Further external experiments are needed to verify this conjecture.

C-C motif ligand CCL4, CCL5 belong to CC family chemokine. CCL4, CCL5, CXCL9, CXCL10 were confirmed to be preferentially expressed in tumors that contained T cells [38]. This suggests that CCL4, 5 and CXCL9,10 may act in the same pathway in T-cell recruitment. The experimental results carried out by Michelle H et al. showed that expression of CXCR3 ligands and CCL5 in chemotherapy tumors did lead to

a synergistic increase in T-cell infiltration [39]. In our study, these four genes were mainly enriched in cytokine-cytokine receptor interaction, chemokine signaling pathway and toll-like receptor signaling pathway. The expression of CCL4, CCL5 showed positive correlation with multiple immune cells infiltration (B-cell, CD8+ T cells, CD4+ T cells, macrophages, Neutrophils, Dendritic cells). It is also been reported that CCL5 released by tumor cells acts its function via paracrine signaling to attract NKs to the tumor bed [40]. However, previous studies showed that tumor growth is delayed in CCR5-/- mice, associated with reduced tumor regulatory T cells infiltration [41]. Edwin L et al. found that a selective increase in CXCL10, CCL4, and CCL17 may contribute to aggressive development of brain metastasis [42]. In the present study, high expression of CCL4 and CCL5 associated with better overall survival and were found to be closely related to stage I melanoma patients compared to other stages. Taken together, we assumed that CCL4 and CCL5 may be potent biomarkers and targets for melanoma.

Neuromedin U (NMU), a neuropeptide belonging to the neuromedin family, is involved in stress, obesity, immune, and energy metabolism [43]. A recent study found the YAP1–NMU axis is associated with pancreatic cancer progression and poor prognosis [44]. High expression of NMU was found to be related to reginal metastasis of head and neck squamous cell carcinoma [45]. NMU upregulation in the breast cancer tissues was proposed as prognostic biomarker for poor outcome, but only in HER2-overexpressing tumor [46]. Moreover, patients with NSCLC (non-small-cell lung carcinoma) and NMU-positive tumor showed significantly shorter survival times than patients with NMU-negative tumor [47]. All these findings suggest that NMU is associated with cancer development and NMU may serve as a new biomarker of poor prognosis. Furthermore, NMU has been proved to be an important resistance-enhancing factor. Overexpression of NMU in sensitive cells makes it resistant to the tested drugs, while NMU silencing sensitized resistant cells [46]. Therefore, silencing NMU can be an effective way for cancer treatment. Researchers found the results in proliferation and survival of NMU appeared to be cancer-type dependent [48,49]. However, research on melanoma and NMU is still lacking. In our study, high expression of NMU result in poor overall survival. NMU receptors are expressed on macrophages and endothelial cells, indicating that NMU is highly related to the tumor microenvironment [50]. Therefore, NMU is a potential tumor growth and progression biomarker in melanoma and silencing NMU may be an effective way for melanoma treatment. More research is needed to reveal its role in melanoma microenvironment.

Galanin and GAMP prepropeptide (GAL) is a neuropeptide, which is found to be expressed in several cancers such as gastrointestinal cancer, head and neck squamous cell carcinoma, brain tumor and salivary duct carcinoma [51]. Galanin acts its function through binding to three receptors (GALR1, GALR2, GALR3). The activation of GALR1 is usually anti-proliferation, while the activation of GALR2 may have the effect of promoting proliferation or anti-proliferation [52]. GALR1 and GALR2 inhibit proliferation of head and neck squamous cell carcinoma (HNSCC) cells. Hypermethylation of GALR1, GALR2 is associated with poor survival and high recurrence in HNSCC [53]. Therefore, galanin and galanin receptors (GALRs) are gradually used as new biomarkers for prognosis in HNSCC. Another research found that the down-regulation of Galanin and GALR1 is related to development of gastric cancer [54]. Besides, Galanin and GAL receptors have been found to be expressed in human skin [55]. Few studies have proved the role of GAL in melanoma. In our results, overall survival of samples with high expression

of GAL showed in poor outcome. Four drugs interacted with GAL in the predicted drug-gene interaction. The relationship between GAL, GALRs and melanoma remains unknown, and more research is needed to verify it.

This study has several limitations. First, the microchip data were acquired from a relatively small number of melanomas deposited in a public database, and only 472 patients with corresponding transcriptome data were acquired from the TCGA cohort. Second, further studies, including in vivo and in vitro experiments as well as validated cohorts, are needed to verify the absoluteness of these findings.

Conclusion

In summary, the present study identified several hub genes, including CCL4, CCL5, NMU, GAL, CXCL9, CXCL10 and CXCL13, which were significantly associated with tumor progression, overall survival and immune infiltration, and might assist in better understanding of the underlying carcinogenesis or advancement for SKCM. This study provided novel insights into the prognosis and therapy of melanoma patients, while further studies are required to elucidate molecular pathogenesis and alteration in signaling pathways of these genes in SKCM.

Abbreviations

SKCM: skin cutaneous melanoma; GEO: gene Expression omnibus; DEGs: differentially expressed genes; PPI: protein-protein interaction; MCODE: Molecular Complex Detection; GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; PM: primary melanoma; NS: normal skin; TCGA: The Cancer Genome Atlas; DGIdb: Drug–Gene Interaction database; NSCLC: non-small-cell lung carcinoma; HNSCC: head and neck squamous cell carcinoma; GALRs: galanin receptors; IHC: immunohistochemistry.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets supporting the conclusions of this article are available in the GEO (<https://www.ncbi.nlm.nih.gov/geo/>) and TCGA repository (<https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/studied-cancers>).

Competing interests

The authors declare that they have no competing interests

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No.

Authors' contributions

SGL initiated the study and organized; HB and HW designed and carried out bioinformatics analyses, statistical analyses, drew figures and drafted the manuscript; SZF participated in modifying the manuscript. All authors read and approved the final manuscript.

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Table 1. Clinicopathological characteristics of SKCM patients

Characteristics	GEO cohort (N=93)	TCGA cohort (N=472)
N (%)		
Age		
≤60 years	53(56.9)	258(54.7)
>60 years	40(43.1)	214(45.3)
Gender		
Male	55(59.1)	297(61.9)
Female	38(40.9)	183(38.1)
Clark level		
I	13(13.9)	6(1.8)
II	25(26.8)	18(5.5)
III – IV	47(50.7)	246(75.5)
V	8(8.6)	56(17.2)
Breslow depth(mm)		
≤0.75	15(16.1)	36(10.2)
0.76-1.50	30(32.2)	65(18.4)
1.51-4.00	39(41.9)	106(30.0)
≥4.00	9(9.8)	146(41.4)
pT stage		
T1-T2	57(61.3)	121(32.7)
T3-T4	36(38.7)	249(67.3)
pN stage		
N0	93(100)	236(65.0)
N1	0(0)	75(20.7)
N2	0(0)	52(14.3)
pM stage		
M0	93(100)	424(94.4)
M1	0(0)	25(5.6)
Pathologic stage		

I- II	93(100)	233(53.9)
III-IV	0(0)	199(46.1)
Persistent distant metastasis		
No	93(100)	217(46.1)
Yes	0(0)	254(53.9)

SKCM: Skin Cutaneous Melanoma

GEO: the Gene Expression Omnibus

TCGA: the Cancer Genome Atlas

Figures

Figure 1

Flowchart for this study. GEO: gene Expression omnibus; DEGs, differential expression genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; TCGA, The Cancer Genome Atlas.

Figure 2

Venn diagram, PPI network and the most significant module of DEGs. a, the three datasets explored an overlap of 308 genes in Venn diagram. b, the PPI network of DEGs was performed by Cytoscape. c, the most significant module of DEGs was obtained from PPI network with 12 nodes. (DEGs, differentially expressed genes; PPI, protein-protein interaction)

Figure 3

GO and KEGG analysis of the overlapping DEGs in melanoma. a, Biological process. b, Cellular component. c, Molecular function. d, KEGG pathway. Functional and pathway enrichment of DEGs was performed by DAVID, displayed in bubble charts.

Figure 4

The KEGG pathway analysis of hub genes via ClueGO ($P < 0.01$). a, the most significant pathway and related genes. b, the number of genes involved in each pathway. c, the classification pie charts were listed as follows: 80.00% terms belong to interleukin-10 signaling and 20.00% terms belong to peptide ligand-binding receptors.

Figure 5

Univariate survival analysis of the hub genes was performed using the Kaplan-Meier curve. The 7 genes of 12 hub genes showed significant difference in overall survival ($P < 0.01$).

Figure 6

Assessment of diagnostic value by ROC curves. Results showed CCL4(AUC=0.872), CCL5(AUC=0.775), CXCL9(AUC=0.834), CXCL10 (AUC=0.786), CXCL13 (AUC=0.807), NMU(AUC=0.829) except GAL(AUC=0.536) were of modest diagnostic value with p value<0.001.

Figure 7

a, CXCL9, 10, 13 and CCL4, 5 were significantly overexpressed in stage 0 and decreased in subsequent stages (P value<0.001).

Figure 8

The expressions of CXCL9, 10 and CCL4 ($p < 0.05$) were negatively correlated with Breslow depth from (0-1.5mm) to (>3mm) while NMU, GAL ($P < 0.05$) were positively correlated with it.

Figure 9

GEPIA database based on TCGA cohort indicated that CXCL9, 10, 13 and CCL4, 5, NMU were significantly high expressed in tumor tissue compared to normal $p < 0.01$.

Figure 10

IHC results revealed CCL4, 5, CXCL9, 10, 13 and NMU were detected in melanoma tissues while not detected in normal tissues using online database.

Figure 11

a, There was a positive correlation between CXCL9 expression and the infiltration of B cells (Cor=0.238, p=3.58e-07), CD8+T cells (Cor=0.643, p=1.94e-52), CD4+T cells (Cor=0.299, p=1.19e-10), macrophages (Cor = 0.247, p = 9.59e-8), neutrophils (Cor = 0.612, p = 7.03e-48), and dendritic cells (Cor = 0.648, p = 1.56e-54; Figure 9a). b-e, Similar results were obtained for CXCL10, CXCL13, CCL4 and CCL5. f-g, While GAL was found negatively correlated with infiltration of CD8+T cells (Cor= -0.171, p=3.22e-4), CD4+T

cells (Cor= -0.108, $p=2.30e-2$), neutrophils (Cor = -0.185, $p = 7.94e-5$), and dendritic cells (Cor = -0.135, $p = 4.42e-3$; Figure 11g).

Figure 12

a, CXCL9 significantly correlated with abundance of 28 types TILs such as activated CD8 T cells (Act_CD8 T cells; rho = 0.801, $p < 2.2e-16$), regulatory T cells (Treg; rho = 0.693, $p < 2.2e-16$), macrophage (rho = 0.655, $p < 2.2e-16$), natural killer T cells (NK T cells; rho = 0.74, $p < 2.2e-16$), myeloid derived suppressor cells (MDSC; rho = 0.744, $p < 2.2e-16$), immature B cell (Imm_B; rho = 0.78, $p < 2.2e-16$). b-e, Similar results were found in CXCL10, CXCL13, CCL4 and CCL5. f-g, NMU, GAL were negatively correlated with abundance of TILs.

Figure 13

Drug–gene interaction diagram, yellow circle indicates the related hub gene and white square indicates the drug.

Supplementary Files

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