Genomic analyses identify signaling pathways and biological processes in small and large melanoma

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Abstract

Melanoma is a skin malignancy that involves different signaling pathways with different sizes and phenotypes. However, the mechanism and function of small and large melanoma are unknown. Here, the aim of this study is to identify the functional molecules and signaling pathways by analyzing the RNA-seq data. The GSE164357 was created by the Illumina MouseWG-6 v2.0 expression bead chip. The KEGG and GO analyses indicated that Antigen processing and presentation and Phagosome are the main processes between the large and small melanoma. Moreover, we identified ten interactive molecules including STAT1, TTN, CALR, ACTA1, FN1, TNNT3, MYL1, TNNI2, RHOA, and TCAP. Therefore, our study may help to further understand the mechanism of melanoma.

Introduction

Melanomas originate from the melanocytes, which colonize the skin and a broad range of other tissues¹. Most melanomas can be found on sun-exposed skin². Different melanomas differ in their anatomical site of origin, and types of oncogenic alteration³. Melanomas originate from the head and neck of aging people who have a high mutation burden⁴. Melanomas affect different signaling pathways that govern proliferation, growth and metabolism, cell identity, resistance to apoptosis, and cell cycle control⁵.

Melanoma cells tend to metastasize to the adjacent epidermis, and they also migrate into the normal skin without a correlation with size⁶. Another important step for tumor metastasis is to invade the local lymph nodes, which only can be assessed in the excisions of primary tumors⁷. The vascular invasion of primary melanomas correlated with metastasis to a higher extent than did tumor thickness. Tumors less than 0.76 mm and more than 1.5mm thick usually developed satellite or in-transit metastases, and tumors of an average thickness (0.75–1.5 mm) indicated the highest rate of development of distant metastases⁶. The molecular method not only allows for the assessment of sentinel lymph nodes but also makes it possible to detect cancer cells in the circulation⁸. For example, Melan-A, gp100, and MAGE-3 were used as significant prognostic factors for melanoma cancer⁹.

In our study, we determined the signaling pathways of small and large melanoma cancers by using the RNA-seq data. We identified a number of DEGs and biological processes and created the protein-protein interaction (PPI) network. The DEGs and PPI network may promote the development of melanoma cancer therapy.

Methods

Data resources

Gene dataset GSE164357 was obtained from the GEO database. The data was produced by the Illumina MouseWG-6 v2.0 Gene Expression BeadChip (MD Anderson Cancer Center, 7455 Fannin Street, Houston, TX, USA). The analyzed dataset includes 3 large melanoma tumors and 3 small melanoma tumors.

Data acquisition and processing

The data were organized and conducted by the R package as previously described^{10–15}. We used a classical t-test to identify DEGs with $P < 0.01$ and fold change ≥ 1.5 as being statistically significant.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO)

KEGG and GO analyses were conducted by the R package (clusterProfiler) and Reactome. $P < 0.05$ was considered statistically significant.

Protein-protein interaction (PPI) networks

The Molecular Complex Detection (MCODE) was used to construct the PPI networks. The significant modules were produced from the constructed PPI networks and String networks. The pathway analyses were performed by using Reactome (<https://reactome.org/>), and $P < 0.05$ was considered significant.

Results

Identification of DEGs between small and large melanoma

To study the genetic difference between different tumor sizes, we analyzed the RNA-seq data from small and large melanoma tumors. A total of 599 genes were identified with the threshold of $P < 0.05$. The top up- and down-regulated genes between small and large tumors were identified by the heatmap and volcano plot (Figure 1). The top ten DEGs were listed in Table 1.

Enrichment analysis of DEGs between small and large melanoma

To identify the biological signaling between small and large melanoma tumors, we performed the KEGG and GO analysis (Figure 2). We identified the top ten KEGG pathways including “Epstein–Barr virus infection”, “Phagosome”, “Antigen processing and presentation”, “Cell adhesion molecules”, “Viral myocarditis”, “Hematopoietic cell lineage”, “Allograft rejection”, “Graft–versus–host disease”, “Autoimmune thyroid disease”, and “Asthma”. We then identified the top ten biological processes (BP) including “muscle system process”, “muscle cell differentiation”, “muscle contraction”, “striated muscle cell differentiation”, “response to interferon–gamma”, “antigen processing and presentation”, “cellular response to interferon–gamma”, “antigen processing and presentation of peptide antigen”, “antigen processing and presentation of exogenous antigen”, and “antigen processing and presentation of exogenous peptide antigen”. We identified the top ten cellular components (CC) including “intrinsic component of organelle membrane”, “integral component of organelle membrane”, “myofibril”, “contractile fiber”, “sarcomere”, “intrinsic component of endoplasmic reticulum membrane”, “integral component of endoplasmic reticulum membrane”, “endoplasmic reticulum protein–containing complex”, “I band”, and “MHC protein complex”. We identified the top ten molecular functions (MF) including “actin binding”, “endopeptidase activity”, “actin filament binding”, “cell adhesion molecule binding”, “MHC

protein binding", "peptide antigen binding", "MHC protein complex binding", "MHC class II protein complex binding", "structural constituent of muscle", and "mannosyltransferase activity".

Construction of PPI network between small and large melanoma

To further understand the potential relationships between the DEGs, we constructed the PPI network by using the Cytoscape. The combined score > 0.2 was used as a cutoff to create the PPI network by linking the 452 nodes and 1308 edges. Table 2 presented the top ten genes with the highest degree scores. The top two clusters were shown in Figure 3. We then evaluated the DEGs and PPI by Reactome map (Figure 4) and obtained the top ten significant biological signaling pathways including "Striated Muscle Contraction", "Interferon alpha/beta signaling", "Interferon Signaling", "Cytokine Signaling in Immune system", "Muscle contraction", "Neutrophil degranulation", "Interleukin-10 signaling", "Nectin/Necl trans heterodimerization", "Downstream TCR signaling", and "Post-translational protein phosphorylation" (Supplemental Table S1).

Discussion

Melanoma is a skin malignancy that has increased faster than any other cancer in the United States¹⁶. Currently, efforts have been focused on the genetic changes that promote melanoma metastasis¹⁷. Thus, our study aims to identify the genetic difference between different sizes of melanoma to explore the potential treatment.

By KEGG and GO analyses, we found that antigen processing and presentation and phagosome are the main processes between large and small melanoma. The study by Shashank J Patel et al found the relationship between the effector function of CD8 + T cells and resistance of cancer immunotherapies¹⁸. Rebecca C Obeng et al found that the phosphopeptide-specific T cell lines can be used as a tool for the treatment of melanoma¹⁹. Similarly, Zhi Xia et al found that the phagosome signaling pathway is the major player in mouse melanoma²⁰.

In this study, we discovered the potential interacting molecules by using the PPI network. The study by Michaël Cerezo et al found that there is a translational regulation of tumor immune escape via the eIF4F-STAT1-PD-L1 axis in melanoma²¹. G protein-coupled receptors (GPCRs) and RGS are involved in the regulation of various biological processes including inflammation, metabolism, cell survival, proliferation, and migration²²⁻³³. Interestingly, Stéphane Pelletier et al found that GPCR agonists promote the tyrosine phosphorylation of STAT1 in a Rac-dependent manner³⁴. Ying Wang et al found TTN-AS1 promotes metastasis by maintaining TTN expression in melanoma³⁵. The study by Ruo Chen et al found that the CALR-TLR4 complex represses the lung cancer progression by mediating the migration and maturation of dendritic cells³⁶. ACTA1 was found as a strong association molecule in the progression of prostate cancer with PTEN mutation³⁷. The downregulation of FN1 can repress colorectal cancer by inhibiting proliferation, migration, and invasion³⁸. The study by Sara Lindström et al found that TNNT3 has a

strong relationship with breast cancer³⁹. The study by Zihang Chen et al found that MYL1 is a significant gene marker for rhabdomyosarcoma⁴⁰. The study by Koichi Sawaki et al found that TNNI2 is considered a specific biomarker for the prediction of gastric cancer metastasis⁴¹. The downregulation of RhoA promotes breast cancer metastasis with increased CCR5 and CXCR4 chemokines signaling⁴². Circadian gene clocks control the downstream genes and targets to regulate the physiological and pathological processes including cancer, aging, immunity, and metabolism^{43–53}. Interestingly, the CLOCK and BMAL1 can activate RHOA to drive the F-actin formation in cancer cells⁵⁴. TCAP has been implicated in the progression of tumor-associated with reproductive tissues⁵⁵.

In conclusion, our study discovered the significant genes between large and small melanoma. The Antigen processing and presentation and Phagosome are the main impacted processes. Therefore, this study may be beneficial to the treatment of melanoma.

Declarations

Author Contributions

Zhiwen Qian, Tingxiang Chang, Tingting Zhang: Methodology. Hanming Gu, Jing Wang: Conceptualization, Writing and Editing.

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Declarations of interest

There is no conflict of interest to declare.

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Tables 1-2

Tables 1-2 are available in the Supplementary Files section.

Figures

Figure 1

Heatmap and volcano plot were created between the small and large melanomas

(A) Heatmap of significant DEGs. Significant DEGs ($P < 0.01$) were used to produce the heatmap.

(B) Volcano plot for DEGs between the small and large melanoma. The most significantly changed genes are highlighted by grey dots.

Figure 2

KEGG and GO analyses of DEGs between small and large melanomas

(A) KEGG analysis, (B) Biological processes, (C) Cellular components, (D) Molecular functions.

Figure 3

The PPI network analyses of DEGs between small and large melanomas

The cluster (A) and cluster (B) were constructed by MCODE.

Figure 4

Reactome map representation of the significant biological processes between small and large melanomas

Supplementary Files

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