DERMATOLOGICA SINICA 34 (2016) 14–19

Contents lists available at ScienceDirect

Dermatologica Sinica

journal homepage: http://www.derm-sinica.com

ORIGINAL ARTICLE

Prediction of the engendering mechanism and specific genes of primary melanoma by bioinformatics analysis

Lei Wu [1, 2], Bin Dong [2], Fang Zhang [3], Yonglin Li [2], Linbo Liu [1, *]

[1] Department of Plastic Surgery, First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[2] Department of Plastic Surgery, The No. 1 People's Hospital of Zhengzhou, Zhengzhou, China
[3] Department of Plastic Surgery, Zhengzhou Central Hospital, Zhengzhou, China

ARTICLE INFO

Article history:
Received: Feb 5, 2015
Revised: Jun 4, 2015
Accepted: Jul 25, 2015

Keywords:
differentially expressed gene
melanoma
protein–protein interaction network
transcription factor
tumor-associated gene

ABSTRACT

Objective: Our aim was to explore the engendering mechanism and gene targets for melanoma.
Methods: The microarray data of GSE46517 were downloaded from the Gene Expression Omnibus database. The differentially expressed genes (DEGs) between primary melanoma samples and normal controls were analyzed using the GEO2R online tool. The screened DEGs were mapped to a protein–protein interaction network based on the Search Tool for the Retrieval of Interacting Genes database. The functions and pathways involved with DEGs were analyzed using the Database for Annotation Visualization and Integrated Discovery software) online tools. Then, the DEGs were further annotated via the TRANSFAC, Tumor-Suppressor Gene, and Tumor-Associated Gene databases.
Results: A total of 1095 DEGs including 511 upregulated genes and 584 down-regulated ones were screened out. The nodes of *CCL5*, *ISG15*, *CDKN2A*, *EGFR*, and *ERBB2* showed a high connectivity degree in protein–protein interaction networks and were mainly enriched in Biological Process GO terms such as the regulation of catalytic activity and cell adhesion, as well as the pathways of cytochrome P450. The DEGs were classified into 31 transcription factors and 43 downregulated tumor associated genes.
Conclusion: Catalytic activity, cell adhesion, and the cytochrome P450 associated pathways are dysregulated in the melanoma formation. The significant nodes such as *ISG15*, *IRF4*, *ERBB2* and *EGFP* may be potential targets for primary melanoma treatment.

Introduction

Melanoma is a type of skin cancer characterized by the neoplasm development of pigment-producing cells called melanocytes. Melanoma is common in the skin, but may also develop in the eyes, ears, gastrointestinal tract, leptomeninges, oral and genital mucous membranes.[1] Melanoma is among the most rapidly increasing cancers of the white population in the USA.[2] It is estimated that 61,300 new cases are expected to be diagnosed in 2013.[3] Melanoma has metastatic potential and the median survival for metastatic melanoma patients is only 6–9 months.[4] Melanoma poses a burden to public health. A good understanding of genetic events underlying melanoma development can lead to the success of targeted therapies in melanoma treatment.[5] Melanoma pathogenesis has been proved to be driven by genetic mutation. Curtin et al[6] indicated that the mutations of *BRAF* or *NRAS* are common in the majority of melanomas without chronic sun-induced damage in skin. Reports showed that *MITF*, *CCND1*, *BRAF*, *CDKN2A*, and *PTEN* are some of the validated oncogenes and tumor suppressors selectively targeted by focal copy number-changing aberrations in melanoma.[7–9] Furthermore, microarray technology has been applied for investigating gene expression patterns for melanoma. Lawrence et al[10] developed microarray data of GSE46517 and proposed that chromosome 10, which encodes multiple tumor-suppressive functions, is frequently lost in human melanoma.[10] With the microarray data of GSE8041 and GSE46517, McCorkle et al[11] found that NME1 regulated the expression of genes that related with the metastasis and outcomes of patients with melanoma. However, the systematic studies of the aberrant gene expression of melanoma

Conflicts of interest: The authors declare that they have no financial or non-financial conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Department of Plastic Surgery, First Affiliated Hospital of Zhengzhou University, Zhengzhou 450000, China.
E-mail address: LinboLiulbl@163.com (L. Liu).

http://dx.doi.org/10.1016/j.dsi.2015.07.003

are rare and the molecular mechanism of melanoma development remains unclear.

Therefore, in the present study, we downloaded the publicly available microarray data (GSE46517) from the Gene Expression Omnibus (GEO) database and performed comprehensive analysis of gene expression patterns. The aim of this current work was to explore the potential molecular mechanism of melanoma and uncover the candidate gene targets for melanoma treatment.

Methods

Gene expression profiles

The publically available dataset (accession number: GSE46517) were downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/).[12] The expression profiles data were developed from 121 samples, including 73 metastatic melanoma samples, 31 primary melanoma samples, one epithelial melanocyte sample, and seven normal skin samples. In the present work, the data from the 31 primary melanoma samples and seven normal skin samples were selected for further analysis based on the Affymetrix Human Genome U133A 2.0 Array (HG-U133A_2) platform.

Differentially expressed gene screening

The differentially expressed genes (DEGs) between primary melanoma and the normal controls were analyzed using the web tool GEO2R (https://www.ncbi.nlm.nih.gov/geo/info/geo2r.html). Differences in gene expression values were evaluated by t test. Multiple testing correction was performed using the Benjamini–Hochberg method.[13] Finally, the DEGs with t ($\log_2$ fold change) >1 and adjusted $p < 0.05$ were screened.

Protein–protein interaction network construction

The Search Tool for the Retrieval of Interacting Genes (STRING) database is a collection of protein interaction data. It provides function and scores for protein pairs.[14] The protein interaction pairs of the screened DEGs were analyzed via the STRING database. Protein pairs with required confidence (combined score) >0.9 were screened for protein–protein interaction (PPI) network construction. The network was visualized by Cytoscape software.[15] The hub nodes in the PPI network were then identified based on the connectivity degree (number of neighbors) in the network statistics according to the scale-free attribute.[16]

Functions and pathways enrichment of DEGs

The Gene Ontology (GO) database is an open source for function annotation of a scale of genes.[17] The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database is a collection of various biochemical pathways.[18] The significantly overrepresented GO terms in biological process (BP) and pathways of DEGs were analyzed using the database for annotation, visualization and integrated discovery.[19] A p value < 0.05 was set as cut-off criterion.

Gene function annotation

The potential transfer factors (TFs) among DEGs were screened based on the TRANSFAC database.[20] The oncogenes and anti-oncogenes of the DEGs were determined based on the Tumor-Suppressor Gene (TSGene)[21] and Tumor-Associated Gene (TAG) databases according to the method described by Chen et al.[22]

Results

DEG screening

A total of 1095 genes corresponding to 1349 probes were identified to be differentially expressed, which included 511 upregulated DEGs and 584 downregulated genes. The detailed information is listed in Table 1.

PPI network

The PPI networks for upregulated and downregulated DEGs were constructed respectively. The PPI network for upregulated genes contained 146 nodes and 278 edges (Figure 1A). As shown in Figure 1B, there were 194 nodes and 265 edges in the PPI network for downregulated genes. After the connectivity degree analysis, the top 20 nodes with high degrees for the up- and downregulated PPI network were screened (Table 2). The connectivity degrees of the Top 20 nodes in the upregulated and downregulated PPI were all higher than 6.

Functions and pathways enrichment analysis of DEGs

To explore the functions of the DEGs, the 511 upregulated and 584 downregulated genes were subjected to GO-BP and KEGG pathway enrichment analysis. As shown in Table 3, the significantly enriched GO terms for upregulated genes were mainly related with the regulation of catalytic activity (GO:0050790), innate immune response (GO:0045087), chemotaxis (GO:0006935), and the regulation of immune response (GO:0050776). The downregulated genes were mainly enriched in cell adhesion (GO:0007155), response to wounding (GO:0009611), the positive regulation of gene expression (GO:0010628), and the enzyme linked receptor protein signaling pathway (GO:0007167). KEGG pathway analysis (Table 4) showed that upregulated genes were mainly enriched in the chemokine signaling pathway (04062), the pathways in cancer (05200), and the cytokine–cytokine receptor interaction (04060). The significantly enriched pathways of downregulated genes were metabolic pathways (01100) and focal adhesion (04510). Notably, pathways of drug metabolism–cytochrome P450 and metabolism of xenobiotics by cytochrome P450 were enriched by the downregulated DEGs.

Functional annotations of DEGs

In order to analyze the aberrant gene expression pattern for TFs and TAGs in primary melanoma, further functional analysis and annotation for DEGs were performed. As shown in Table 5, 12 upregulated DEGs (such as *MITF*, *CDK2*, *STAT1*, *IRF4*, and *IRF7*) were identified to be TFs and 19 downregulated DEGs that determined to be TFs included *EGR3*, *SMARCA2*, *TBX3*, *VDR*, and *RXRA*. Furthermore, based on the TAG database, 20 upregulated DEGs were identified to be well-documented TAGs, of which *CCND2*, *CEP55*, *FOSL1*, and *IRF4* were oncogenes, and *MMP11*, *CDKN2A*, *LPL*, *EIF2AK2*, *MAD1L1*,

Table 1 The numbers of the upregulated and downregulated probes and differentially expressed genes.

	Probe counts	Gene counts
Upregulated	607	511
Downregulated	742	584
Total	1349	1095

Probe counts = number of probes with query signal; Gene counts = number of differentially expressed genes.