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Screening genes associated with melanoma using a combined analysis of mRNA and methylation microarray

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ABSTRACT

As an aggressive skin cancer with high morbidity and mortality, melanoma is becoming a heavy burden on society. This study is aimed to identify genes associated with melanoma. Both expression profile of GSE31909 and methylation profile of GSE53801 were downloaded from Gene Expression Omnibus. GSE31909 included 3 melanoma cell line SK-MEL-28 samples, 3 melanoma cell line LOXIMVI samples, 3 normal melanocyte line HEMn samples and 3 normal melanocyte line HEMa samples. Meanwhile, GSE53801 included 2 human melanoma cell lines and 8 normal human epidermal melanocytes. Differentially expressed genes (DEGs) and differentially methylated sites were screened using limma package in R. Besides, common genes of the DEGs and the genes with differentially methylated sites were identified. Afterwards, enrichment analyses were performed for the DEGs and the common genes using DAVID online tool and the cytoscape plug-in clueGO + cluePedia, respectively. Moreover, transcription factor (TF)–mRNA pairs were searched and then TF–mRNA regulatory network was visualized by Cytoscape. Additionally, the DEGs were validated in melanoma cell lines A375 samples from GSE68453. Total 609 DEGs and 5893 differentially methylated sites in melanoma cell lines compared with normal melanocytes were screened. And 140 common genes (e.g. *CTGF*, *PLXNB1*, *CD9*, *ADM* and *EPHA4*) were identified. Several functions were enriched, including response to wounding and integrin binding. Meanwhile, the TF–mRNA regulatory network involved several transcription factors and their target genes (e.g. *LDOC1*, *CTGF*, *STAT3*, *EGR1*, *SETDB1* and *C-MYC*), which might function through interacting with each other (e.g. *STAT3* → *CTGF*). These genes might play important roles in melanoma progression.

1. Introduction

Melanoma is an aggressive skin cancer that has high morbidity and mortality (Tuong et al., 2012). In the United States, melanoma is the fifth most common malignancy in men, and the sixth most common malignancy in women (Chen et al., 2013). There are approximately 9180 patients who died from melanoma in 2012 in the United States (Society, 2012). And the major risk factor for the progression of melanoma is ultraviolet radiation (Wang et al., 2001). As the deadliest form of skin cancer (Shoo and Kashani-Sabet, 2009; Chen et al., 2013), melanoma is becoming a heavy burden on society (Linos et al., 2009).

Recently, several studies have been performed to investigate the influences of genes and transcription factors (TFs) on melanoma. For example, chondroitin sulfate proteoglycan 4 (CSPG4), which is a highly immunogenic tumor antigen, is implicated in formation and poor prognosis of melanoma tumor (Price et al., 2011). Inactivation of tumor suppressor gene *p16* may be critical in the development of acral lentiginous melanomas (Chana et al., 2000). Melanoma differentiation-associated gene-7 (*mda-7*) regulates induction of the GADD family of genes via the p38 mitogen-activated protein kinase (MAPK) pathway, which can induce the selective induction of melanoma cell apoptosis (Sarkar et al., 2002). Loss of phosphatase and tensin homolog (*PTEN*) can reduce BIM-mediated apoptosis and promote resistance of intrinsic B-type Raf kinase (BRAF) inhibitor to melanoma cells (Paraiso et al., 2011). By inhibiting forkhead box O (*FOXO*), tribbles homolog 2 (*TRIB2*) contributes to the growth and survival of melanoma cells (Zanella et al., 2010). Expressions of forkhead box O3 (*FOXO3*) and microphthalmia-associated transcription factor (*MITF*) can be inhibited by miR-182 in the progression of melanoma (Segura et al., 2009). As a lineage-determination factor, transcription factor *MITF* is essential for melanoma clonogenic growth disrupted by β-catenin–T-cell transcription factor/lymphoid enhancer-binding factor (LEF) (Widlund et al., 2002).

In 2014, Dahl et al. performed a whole-genome screen for abnormal promoter methylation in melanoma, and identified the hypermethylation of KIT promoter not only in melanoma cell lines, but also in primary and metastatic cutaneous melanomas (Dahl et al., 2015). In the study, to further unravel the molecular mechanisms of melanoma, we performed a more comprehensive bioinformatics analysis of mRNA and methylation microarray. Briefly, we identified differentially expressed genes

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(DEGs) and differentially methylated sites in melanoma cell lines compared with normal melanocytes. Then, potential functions of the DEGs were analyzed by functional and pathway enrichment analyses. Besides, common genes of the DEGs and genes with differentially methylated sites were obtained. In addition, TF–mRNA pairs were searched and TF–mRNA regulatory network was constructed. What's more, the DEGs were validated in melanoma cell lines A375.

2. Materials and methods

2.1. Microarray data

Expression profile of GSE31909 and methylation profile of GSE53801 were downloaded from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/). GSE31909, which was sequenced on the platform of GPL10558 Illumina HumanHT-12 V4.0 expression beadchip, included 3 melanoma cell line SK-MEL-28 samples, 3 melanoma cell line LOXIMVI samples, 3 normal melanocyte line HEMn samples and 3 normal melanocyte line HEMa samples. GSE53801, which was based on the platforms of GPL8169 Agilent-014706 Human Promoter ChIP-on-Chip Set 244k, Microarray 1 of 2 G4489A (Probe name version) and GPL8170 Agilent-014707 Human Promoter ChIP-on-Chip Set 244k, Microarray 2 of 2 G4489A (Probe name version), included 2 human melanoma cell lines and 8 normal human epidermal melanocytes.

2.2. DEGs and differentially methylated site screening

After GSE31909 and GSE53801 were downloaded, microarray data were preprocessed using affy package (Gautier et al., 2004) in Bioconductor. At first, background correction, normalization and Log2 conversion were successively performed to obtain standardized expression matrix. Afterwards, probe symbols were converted to gene symbols. At last, the average value of multiple probes corresponding to one gene was obtained as final gene expression value. The linear models for microarray data (limma) package (Diboun et al., 2006) in R were used to screen DEGs and differentially methylated sites in melanoma cell lines compared with normal melanocytes. The adjusted p-value < 0.05 and $|\log_2$fold-change (FC)$| > 1$ were used as the cut-off criteria. Using the annotation information of methylation profile, genes corresponding to the differentially methylated sites were obtained. Subsequently, the DEGs were compared with the genes with differentially methylated sites to obtain their common genes.

2.3. Functional and pathway enrichment analysis

As a web-accessible program, Database for Annotation, Visualization and Integrated Discovery (DAVID) integrates intuitive graphical summaries with functional genomic annotations (Dennis et al., 2003). Gene Ontology (GO) terms are used to describe molecular function (MF) of genes, the biological process (BP) in which they involved, as well as their cellular component (CC) (Tweedie et al., 2009). Kyoto Encyclopedia of Genes and Genomes (KEGG) is a comprehensive database used for understanding higher order functional meanings (Kanehisa et al., 2002). By DAVID online tool, functional and pathway enrichment analyses were conducted for DEGs in melanoma cell lines compared with normal melanocytes. The p-value < 0.05 and involving at least 10 genes were used as the cut-off criteria. Using the cytoscape plug-in clueGO + cluePedia (Bindea et al., 2013), GO functional and KEGG pathway enrichment analyses were also performed for the common genes. The adjusted p-value < 0.05 was used as the cut-off criterion.

2.4. TF–mRNA regulatory network construction

After the methylated sites on the common genes were obtained, combining with the binding site information of transcriptional factors (TFs) in UCSC database (Fujita et al., 2010) and chromosome location information of the methylation sites, TF–mRNA pairs were searched. The genes, in which differentially methylated sites overlapped with binding sites of TFs, were selected as the target genes of the TFs. Then, the Cytoscape software (Shannon et al., 2003) was used to visualize the TF–mRNA regulatory network.

2.5. Validation of the DEGs in melanoma cell lines A375

Expression profile of GSE68453 was also downloaded from GEO, which was based on the platform of GPL10558 Illumina HumanHT-12 V4.0 expression beadchip. And data of melanoma cell lines A375, which included 18 drug antagonism samples and 6 control samples, were selected from GSE68453 to validate the screened DEGs. Using the GEO2R (http://www.nci.nlm.nih.gov/geo/geo2r/) (Barrett, 2013) online tool, the DEGs between drug antagonism samples and control samples were identified, with p-value < 0.05 as the threshold.

3. Results

3.1. DEGs and differentially methylated site analysis

A total of 609 DEGs were screened in melanoma cells compared with normal melanocytes, including 171 up-regulated genes and 438 down-regulated genes. There were more down-regulated genes compared with up-regulated genes. Besides, a total of 5893 differentially methylated sites (which were corresponding to 3532 genes) were identified in melanoma cells in comparison to normal melanocytes, including 555 hypermethylated sites and 5338 hypomethylated sites. Most genes only have 1 differentially methylated sites, and a few genes have more than 5 differentially methylated sites (Fig. 1). There were 140 common genes (e.g. connective tissue growth factor, *CTGF*; plexin B1, *PLXNB1*; CD9 molecule, *CD9*; adrenomedullin, *ADM* and EPH receptor A4, *EPHA4*) between the DEGs and the genes corresponding to the differentially methylated sites. And the common genes contained 260 methylated sites.

3.2. Functional and pathway enrichment analysis

The top 10 GO functions enriched for DEGs in melanoma cell lines compared with normal melanocytes included response to wounding ($p = 1.17E-07$, which involved *CD9*, *CTGF* and *ADM*), membrane-bounded vesicle ($p = 2.39E-08$) and cytoplasmic vesicle ($p = 3.28E-07$) (Table 1A). Meanwhile, pathways such as extracellular matrix (ECM)–receptor interaction ($p = 5.02E-5$), lysosome ($p = 3.73E-3$) and focal adhesion ($p = 7.03E-3$) were enriched for the DEGs in melanoma cell lines compared with normal melanocytes (Table 1B).

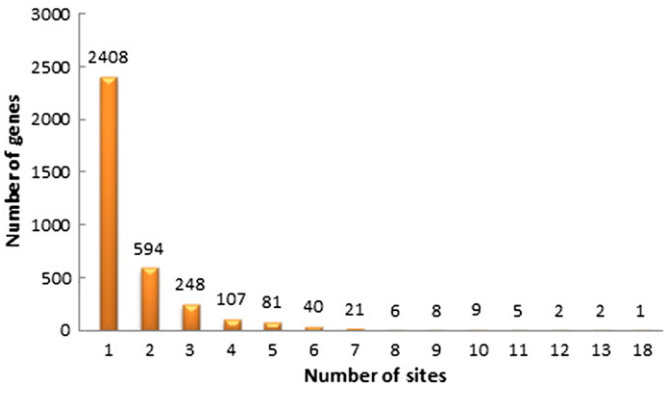

Fig. 1. The numbers of differentially methylated sites in each gene.