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Genome-wide analysis of aberrant gene expression and methylation profiles reveals susceptibility genes and underlying mechanism of cervical cancer

Q1 Hongmei Lin, Yifei Ma*, Yongqing Wei, Hui Shang

Department of Gynaecology and Obstetrics, Shandong University Affiliated Jinan Center Hospital, Jinan 250013, Shandong, China

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ABSTRACT

Background: This study aimed to explore the molecular mechanism of cervical cancer (CC) by integrated bioinformatic analyses of gene expression and methylation profiles.
Methods: The gene expression and methylation microarrays in CC samples and normal controls were respectively downloaded from the GEO database. After screening the differentially expressed genes (DEGs) with Limma package and the CC-related methylation sites with CpGassoc package in R language, DEGs with CC-related methylation sites were identified from the intersection of the above two groups of results with 50 kb upstream and downstream of a gene as the gene region. Then GO enrichment was performed by GenCLIP2.0 software. Sequentially, analysis of metabolic sub-pathways with pathogenic risk was predicted by iSubpathwayMiner package in R language.
Results: A total of 1357 DEGs including 721 up-regulated and 636 down-regulated, as well as 666 CC-related methylation sites were screened out. After being analyzed, 26 DEGs with 35 CC-related methylation sites were identified. *EDN3* and *EDNRB* were significantly involved in a function cluster in GO terms of vein smooth muscle contraction, vascular smooth muscle contraction and phasic smooth muscle contraction. *LHX2* and *PAX6* were significantly involved in a function cluster in GO terms of telencephalon regionalization and forebrain regionalization. *ACOX3*, *CYP39A1* and *DPYS* were significantly enriched in 25 sub-pathways of 6 major pathways.
Conclusions: *EDN3* and *EDNRB* might play important roles in the molecular mechanism of CC, and *LHX2*, *ACOX3*, *CYP39A1* and *DPYS* might be susceptibility genes and potential risk markers in CC.

Introduction

Cervical cancer (CC) is recently the fifth most frequent cancer among women world-wide [1]. Although the mortality rate of CC is relatively low, its advanced-stage symptoms including abnormal vaginal bleeding, foul-smelling discharge, disruption of normal bladder or bowel function, and leg edema [2] can seriously affect the quality of life and cost a huge sum for its health care, leading a Q2 considerable societal burden.

The aetiological role of infection with high-risk human papilloma viruses (HPVs) in CC is well established. Among more than 200 identified HPV genotypes (http://www.hpvcenter.se/html/refclones.html), the genotype HPV16 is the highest found in CC in Western/Central Asia, HPV18 detected across all regions, HPV45 commonly found in Africa and South/Central America [3]. HPV virus can integrate into the transcribed genomic region of the host genome and this process was suggested as a mechanism of HPV to improve the expression of some viral products, especially E6 and E7 oncogenes [4]. Despite the documented etiologic role of HPV infection, the molecular mechanism in the multistep progression of CC remains largely unknown.

Lately, DNA epigenetic alterations have been paid more and more attention in researches on cancers [5]. It was reported that in CC, increased methylation of the integrated HPV type 16 genes were correlated with a more severe cancer progression [6]. Anomalous methylation status of several host genes related to multiple biological processes including cell cycle, apoptosis, cell adhesion and cellular signaling has also been analyzed [7], suggesting a large variation in DNA methylation frequency in CC.

Recently, studies on gene expression or methylation alterations in CC by high-throughput microarray technology have been reported. Scotto et al. had made gene expression microarrays of

* Corresponding author at: Department of Gynaecology and Obstetrics, Shandong University Affiliated Jinan Center Hospital, No. 105 Jiefang Road, Jinan 250013, Shandong, China.
E-mail address: mayifeiyfm@hotmail.com (Y. Ma).

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CC samples and normal controls and found that a number of functionally important genes such as *E2F1*, *TPX2* and *KIF3B* were found overexpressed as a consequence of the chromosome 20 gain in CC [8]. Also, Farkas et al. have conducted an analysis of methylation microarrays of CC samples and normal controls, which showed an extensive differential methylation signature in CC [9]. They identified candidate biomarker genes for CC which represented several types of mechanisms that are epigenetically deregulated by hypermethylation, such as membrane receptors, intracellular signaling and gene transcription. To explore the potential susceptibility genes and gain a better understanding of the molecular mechanisms in CC, we acquired the mRNA expression microarrays data of Scotto et al. and the methylation microarrays data of Farkas et al. to further conduct a genome-wide integrated analysis on the differentially expressed genes (DEGs) with aberrant methylation between CC samples and normal controls. Various bioinformatics methods were applied to identify the functions and metabolic sub-pathways with pathogenic risk of these genes in CC.

Materials and methods

Data acquisition

The expression data GSE9750 [8] and GSE46306 [9] were downloaded from GEO (Gene Expression Omnibus) database (http://www.ncbi.nlm.nih.gov/geo/).

The GSE9750 data were mRNA expression microarrays based on [HG-U133A] Affymetrix Human Genome U133A Array platform including 66 samples: 33 primary cervical tumors (HPV positive), 9 CC cell lines (HPV positive) and 24 normal cervical epithelium samples (HPV negative). The 33 primary cervical tumor tissue samples were obtained from cervix cancer patients with ages ranged from 28 to 70, and 24 normal cervical epithelium tissue samples were obtained from normal cervix epithelium donors with ages ranged from 27 to 64. The GSE46306 data were methylation microarrays based on GPL13534 (Illumina HumanMethylation450 BeadChip) platform including 44 samples: 20 normal cervical samples (HPV negative), 18 cervical samples with cervical intraepithelial neoplasia grade 3 (CIN3) lesions (HPV positive) and 6 CC tissues (HPV positive). The 6 CC tissues and 20 normal cervical samples were selected and used in this study.

Data preprocessing

The primary probe data were transformed into gene names according to the annotations in corresponding platforms. Then KNN (k-nearest neighbor averaging) rule of the Impute package [10] in R language was applied to interpolate the missing expression values or methylation indexes (k = 10, as the default parameters). The expression level of the gene which responded to multiple probes was defined as the mean of the expression values. Further, microarrays were normalized by the preprocessCore package [11] in R language.

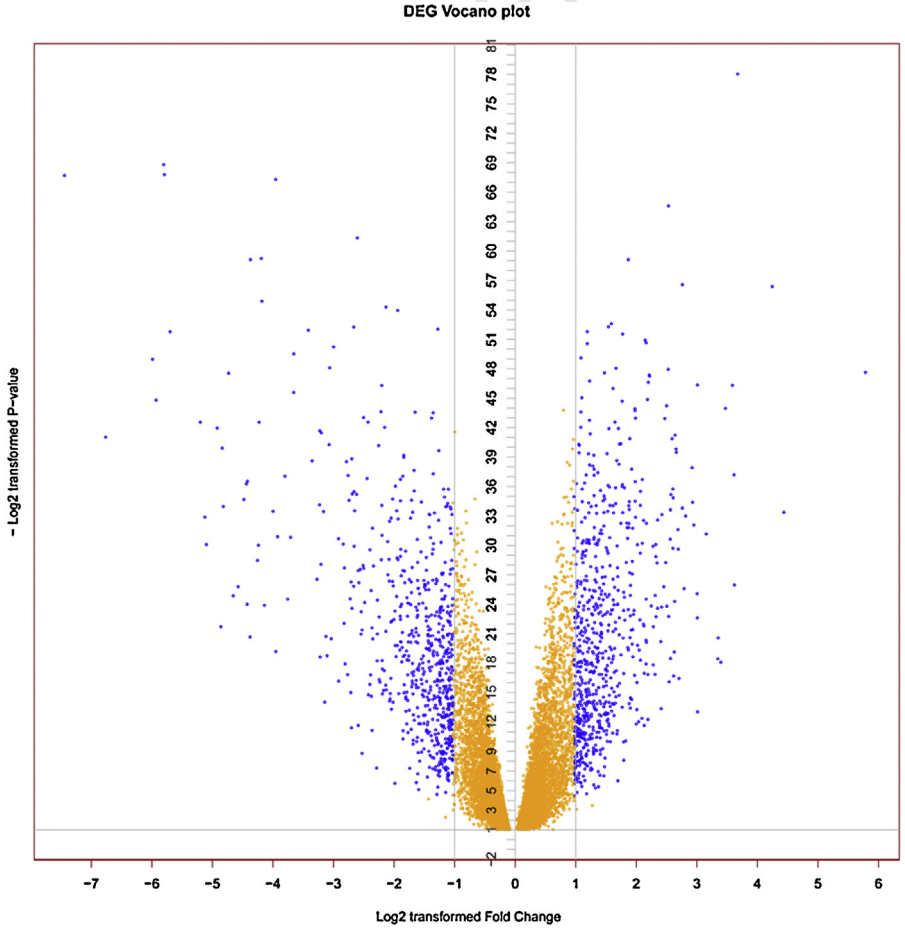

Fig. 1. The volcano plot of differently expressed genes (DEGs) distribution. The vertical axis: $-\log_2$ (transformed P-value); the horizontal axis: $\log_2$ (transformed fold change); blue dots: DEGs; yellow dots: other genes. $|\log_2 FC| > 1$ and adjusted P-value <0.05 were set as cut-off criteria. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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