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Epigenetic regulation on the gene expression signature in esophagus adenocarcinoma

Ting Xi [a], Guizhi Zhang [b,*]

[a] Department of Gastroenterology, People's Hospital of Liaocheng of Shandong Province, China
[b] Department of Gastroenterology, Second People's Hospital of Liaocheng of Shandong Province, China

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ABSTRACT

Background: Understanding the molecular mechanisms represents an important step in the development of diagnostic and therapeutic measures of esophagus adenocarcinoma (NOS). The objective of this study is to identify the epigenetic regulation on gene expression in NOS, shedding light on the molecular mechanisms of NOS.
Methods: In this study, 78 patients with NOS were included and the data of mRNA, miRNA and DNA methylation of were downloaded from The Cancer Genome Atlas (TCGA). Differential analysis between NOS and controls was performed in terms of gene expression, miRNA expression, and DNA methylation. Bioinformatic analysis was followed to explore the regulation mechanisms of miRNA and DNA methylationon gene expression.
Results: Totally, up to 1320 differentially expressed genes (DEGs) and 32 differentially expressed miRNAs were identified. 240 DEGs that were not only the target genes but also negatively correlated with the screened differentially expressed miRNAs. 101 DEGs were found to be highlymethylated in CpG islands. Then, 8 differentially methylated genes (DMGs) were selected, which showed down-regulated expression in NOS. Among of these genes, 6 genes including ADHFE1, DPP6, GRIA4, CNKSR2, RPS6KA6 and ZNF135 were target genes of differentially expressed miRNAs (hsa-mir-335, hsa-mir-18a, hsa-mir-93, hsa-mir-106b and hsa-mir-21).
Conclusions: The identified altered miRNA, genes and DNA methylation site may be applied as biomarkers for diagnosis and prognosis of NOS.

1. Introduction

There are two main kinds of esophageal carcinoma: esophagus adenocarcinoma and esophagus squamous cell carcinoma. Esophagus adenocarcinoma (NOS) is one of the fastest rising cancers. Up to now, the cause of increasing occurrence of NOS is not entirely known but the principal risk factors are male, visceral obesity and chronic reflux disease [1]. Chemotherapy and surgery are common therapeutic methods for patients with NOS. It is pointed out that NOS is resistant to chemotherapeutic regimens and is characterized by a poor survival outcome [2].

Recently, microRNAs (miRNAs) were found to be candidates as biomarkers for diagnosis and prognosis in different cancers [3,4]. MiRNAs are small, well-conserved and non-coding RNA molecules that regulate the expression of mRNAs [5]. Generally, miRNAs inhibit gene expression through targeting the complementary mRNA and blocking its translation [6]. It is well known that miRNAs is tissue-specific in terms of expression and function [7,8]. They can function as oncomir as well as tumor suppressors, and are involved in various biological processes, including proliferation, differentiation, and apoptosis [9]. Some miRNAs have already been identified in NOS, for instance, miR-21, miR-233, miR-192 and miR-194 was up-regulated, and the expression patterns were validated by qRT-PCR [10]. This further suggested that miRNAs plays an important role in the diagnosis and prognosis of NOS.

Epigenetic modifications such as DNA methylation can modify gene expression patterns and control amounts of cellular functions [11]. Aberrant hypermethylation has been associated with inactivation of tumor-related genes in a large-scale of human neoplasms [12]. DNA methylation and miRNA regulation on gene expression both belong to the domain of epigenetic modification. So, it is important to analyze the correlation between transcriptional

* Corresponding author at: Department of Gastroenterology, Second People's Hospital of Liaocheng of Shandong Province, No. 306 JianKang Street, Linqing 252600, China.
E-mail addresses: Piao1130@tom.com (T. Xi), zhangguizhi54321@163.com (G. Zhang).

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sequencing data and epigenetic modification data to illustrate the explicit epigenetic regulation mechanisms of NOS. In this study, the comprehensive analysis of mRNA, miRNA and DNA methylation profiling data of NOS from The Cancer Genome Atlas (TCGA) was performed. Differentially expressed genes (DEGs), differentially expressed miRNAs and differentially methylated genes (DMGs) were identified in NOS. Function enrichment analysis of DEGs and DMGs was then performed. DEGs associated with differential methylation or targeting by differentially expressed miRNAs were identified. At last, 6 candidate genes may be used in the potential targeted treatment of NOS, and further study was needed to investigate their roles in NOS carcinogenesis.

2. Materials and methods

2.1. Datasets

In this study, we downloaded the mRNA expression (UNC IlluminaHiSeqRNASeq), miRNA expression (BCGSC_IlluminaHiSeq_miRNASeq) and DNA methylation data (JHU_USC_HumanMethylation450) of all 185 patients with NOS from TCGA data portal (http://tcga-data.nci.nih.gov/) (January 2016). According to providing clinical information, we selected 78 NOS patients without histories of other malignancy or neoadjuvant treatment. Among which, 68 men and 10 women were included and 79% were the white race. Moreover, the age range of all individuals was 27–86 and the average age is 65. Additionally, the tumor degree was classified as G1 (2 cases), G2 (25 cases), G3 (27 cases) and GX (24 cases). Detailed information of these 78 patients was showed in supplementary 1.

2.2. Analysis of DEGs and differentially expressed miRNAs

The DEGs and differentially expressed miRNAs were evaluated in the R-bioconductor package DESeq [13]. The Limma package in R was used to calculate *p*-values by two-tailed Student's *t*-test. MetaMA package in R was used to combine *p*-values, and the false discovery rate (FDR) was obtained from multiple comparisons using the Benjamini and Hochberg method [14]. We selected DEGs and differentially expressed miRNAs with criterion of FDR < 0.0001.

2.3. Correlation analysis of DEGs and differentially expressed miRNAs

First, pairwise Pearson correlation coefficients between differentially expressed miRNAs and DEGs was calculated, and $P < 0.05$ was defined as statistical significance. Second, six miRNA-target prediction tools (RNA22, miRanda, miRDB, miRWalk, PICTAR2 and Targetscan) were utilized to predict target genes of differentially expressed miRNAs. Only those miRNA-target pairs which were predicted by more than four algorithms can be selected out. The miRNA-targets pairs verified by experiment in miRWalk database were also screened out. Finally, we selected the miRNA-target pairs with negative correlations ($p < 0.05$, $r < 0$) to establish the miRNA-target regulatory network, which was visualized using Cytoscape software [15].

2.4. Functional annotation of target genes of differentially expressed miRNAs

To acquire the biological function of the miRNA target genes, we conducted Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis based on the online software GENECODIS [16]. FDR < 0.05 was set as the cut-off for selecting significantly enriched functional KEGG pathways.

Table 1
NOS patients sample IDs.

Sample ID	Sample ID	Sample ID	Sample ID
TCGA-2H-A9GF	TCGA-JY-A939	TCGA-L5-A4OW	TCGA-L5-A8NW
TCGA-2H-A9GG	TCGA-JY-A93C	TCGA-L5-A4OX	TCGA-L7-A6VZ
TCGA-2H-A9GH	TCGA-JY-A93D	TCGA-L5-A88V	TCGA-M9-A5M8
TCGA-2H-A9GI	TCGA-JY-A93E	TCGA-L5-A891	TCGA-Q9-A6FW
TCGA-2H-A9GJ	TCGA-L5-A43C	TCGA-L5-A893	TCGA-R6-A6DN
TCGA-2H-A9GK	TCGA-L5-A43E	TCGA-L5-A8NE	TCGA-R6-A6DQ
TCGA-2H-A9GL	TCGA-L5-A4OF	TCGA-L5-A8NF	TCGA-R6-A6KZ
TCGA-2H-A9GM	TCGA-L5-A4OH	TCGA-L5-A8NH	TCGA-R6-A6L4
TCGA-2H-A9GN	TCGA-L5-A4OJ	TCGA-L5-A8NI	TCGA-R6-A6L6
TCGA-2H-A9GO	TCGA-L5-A4ON	TCGA-L5-A8NJ	TCGA-R6-A6XG
TCGA-2H-A9GQ	TCGA-L5-A4OO	TCGA-L5-A8NL	TCGA-R6-A6XQ
TCGA-2H-A9GR	TCGA-L5-A4OP	TCGA-L5-A8NM	TCGA-R6-A6Y0
TCGA-IC-A6RE	TCGA-L5-A4OQ	TCGA-L5-A8NN	TCGA-R6-A6Y2
TCGA-IG-A4QS	TCGA-L5-A4OR	TCGA-L5-A8NR	TCGA-R6-A8W5
TCGA-IG-A7DP	TCGA-L5-A4OS	TCGA-L5-A8NS	TCGA-R6-A8W8
TCGA-JY-A6F8	TCGA-L5-A4OT	TCGA-L5-A8NU	TCGA-R6-A8WC
TCGA-JY-A6FH	TCGA-L5-A4OU	TCGA-L5-A8NV	TCGA-R6-A8WG
TCGA-RE-A7BO	TCGA-V5-A7RE	TCGA-VR-A8EQ	TCGA-ZR-A9CJ
TCGA-S8-A6BV	TCGA-V5-AASW	TCGA-VR-AA4D	
TCGA-V5-A7RB	TCGA-V5-AASX	TCGA-X8-AAAR	

2.5. Influence of DNA methylation on DEGs

The COHCAP package in R (https://sourceforge.net/projects/cohcap) [17] was used to identify differential methylation sites between tumors and normal tissues that are more likely to regulate downstream gene expression. FDR < 0.05 was considered as differentially methylated CpG sites. Based on the gene expression of NOS, we identified the aberrant DNA methylated CpG site which affected corresponding gene expression.

3. Results

3.1. Gene expression pattern in NOS

Totally, up to 78 tissue samples were included in the present study, all of which with fully characterized mRNA profiles, miRNA profiles and DNA methylation data. The TCGA barcode IDs for each sample were showed in Table 1. There were 1320 DEGs with FDR < 0.0001 between NOS and normal tissue. Among these genes, 594 genes were up-regulated and 726 genes were down-regulated. The heat map of top fifty DEGs was showed in Fig. 1. The top ten up-regulated genes were ESM1, LOC541471, CKS2, GABRD, MMP11, MMP3, LINC00152, TPX2, IL8 and NUF2. By contrast, the top ten down-regulated genes were LOC388387, GPR155, DQ599327, RGMB, SIK2, AK131020, FAM165B, C22orf23, NDE1 and AK055981.

3.2. Correlations of differentially expressed miRNAs and DEGs in NOS

The screened differentially expressed miRNAs were presented in Table 2. There were 32 differentially expressed miRNAs including 14 up-regulated and 18 down-regulated miRNAs.

To investigate correlations between differentially expressed miRNAs and DEGs, we first performed the correlation analysis. Depending on the analysis, we obtained 3112 miRNA-mRNA pairs which were negatively correlated ($P < 0.05$, $r < 0$). Then, we got 1828 miRNA-mRNA pairs through miRNA-target prediction algorithms. At last, we screened out 240 DEGs including 160 up-regulated and 80 down-regulated genes, all of which were not only negatively correlated with corresponding miRNA expression, but also predicted by bioinformatics methods. The established miRNA regulatory network was showed in Fig. 2.