



Uncovering the potential miRNAs and mRNAs in follicular variant of papillary thyroid carcinoma in the Cancer Genome Atlas database

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Background: Understanding the molecule mechanism is a key step in the development of diagnostic and therapeutic measures of follicular variant of papillary thyroid carcinoma. The objective of this study is to identify differentially expressed miRNAs and mRNAs, shedding light on the molecule mechanism of follicular variant of papillary thyroid carcinoma.

Methods: The data of miRNA, mRNA and DNA methylation were downloaded from The Cancer Genome Atlas (TCGA) database. Differential analysis between the follicular variant of papillary thyroid carcinoma and controls was performed in terms of miRNA expression, mRNA expression and DNA methylation. The regulatory network between miRNAs and mRNAs was constructed followed by the functional analysis of these target mRNAs. Real-time fluorescence quantitative polymerase chain reaction (QRT-PCR) was used to validate the expression of identified miRNAs and mRNAs.

Results: Totally, up to 8 differentially expressed miRNAs, 973 differentially expressed mRNAs and 146 differentially methylated mRNAs were identified. Hsa-mir-222 (degree =33), hsa-mir-221 (degree =29), hsa-mir-214 (degree =13), hsa-mir-138-2 (degree =11) and hsa-mir-34a (degree =4) were miRNAs that regulated the most target mRNAs (such as BCL2, BCL2L11 and PEG3, ALDH1A1, PLA2R1, TFPC2L1, RAB23, TK1 and CTSB). Focal adhesion, MAPK signaling pathway and p53 signaling pathway were three significantly enriched signaling pathways of target differentially expressed mRNAs in the functional analysis. The *in vitro* validation of hsa-mir-222 and hsa-mir-221, CTSB, TFPC2L1 and BCL2 was consistent with the bioinformatics analysis.

Conclusions: The identified altered miRNAs (hsa-mir-222, hsa-mir-221, hsa-mir-214, hsa-mir-138-2 and hsa-mir-34a) and their target mRNAs (BCL2, BCL2L11 and PEG3, ALDH1A1, PLA2R1, TFPC2L1, RAB23, TK1 and CTSB) may be helpful in understanding the molecule mechanism of follicular variant of papillary thyroid carcinoma.

Keywords: Differentially expressed mRNAs; differentially expressed miRNAs; follicular variant of papillary thyroid carcinoma

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Introduction

Thyroid carcinoma, originated from parafollicular or follicular thyroid cells, possesses the highest incidence in the endocrine neoplasias (1). Papillary thyroid carcinoma, medullary thyroid carcinoma, anaplastic thyroid carcinoma, follicular thyroid carcinoma and poorly differentiated thyroid carcinoma is five types of thyroid carcinoma (2). The follicular variant of papillary thyroid carcinoma, a heterogeneous tumor, is the second most common morphologic subtype of papillary thyroid carcinomas (3,4). The follicular variant of papillary thyroid carcinoma has a follicular architecture that is lined by cells with nuclear features of papillary thyroid carcinoma. The follicular variant of papillary thyroid carcinoma includes the encapsulated and infiltrating variants (5). It is hypothesized that the follicular variant of papillary thyroid carcinoma could reflect aggressive parameters including bilateral lesions, vascular invasion, extrathyroid extension and distant metastasis (such as pulmonary metastases and bone metastases) at the time of diagnosis (3,6-9). The prognosis of follicular variant of papillary thyroid carcinoma is favorable and the 10- and 15-year overall survival is 93% and 89%, respectively (10).

MicroRNAs (miRNAs) are small and non-coding single-chained RNAs, which targets mRNAs and causing their translation or degradation (11). Several evidences indicate that miRNAs have crucial functions in adjusting biological behaviors including cell proliferation, apoptosis and differentiation (12,13). It is reported that hsa-mir-146-5p, -146b-3p, -222, -222-5, -221, -551b, -375, 99b-3p and 181-2-3p are deregulated in follicular variant of papillary thyroid carcinoma. Furthermore, hsa-mir-181a-2-3p and hsa-mir-99b-3p are related to the adverse outcome in patients with follicular variant of papillary thyroid carcinoma (14). DNA methylation can modify mRNA expression patterns and aberrant hypermethylation is associated with inactivation of tumor-related mRNAs (15,16). The methylation pattern of NIS, TSHR, SLC26A4, TIMP3, RAR β 2 and RASSF1A has been found in papillary thyroid carcinoma (17-20).

In order to understand the pathology of the follicular variant of papillary thyroid carcinoma, we first performed the comprehensive analysis of miRNA, mRNA and DNA methylation profiling data from the Cancer Genome Atlas (TCGA) database. Differentially expressed miRNAs, mRNAs and methylated mRNAs were identified. Then, we performed the function enrichment analysis of differentially expressed target mRNAs of differentially expressed

miRNAs. Last, we performed the real-time fluorescence quantitative polymerase chain reaction (QRT-PCR) to validate the expression of identified miRNAs and mRNAs.

Methods

Datasets

In this study, we downloaded the miRNA expression (miRNASeq BCGSC IlluminaHiSeq-miRNASeq), mRNA expression (RNASeqV2 UNC IlluminaHiSeq_RNASeqV2) and DNA methylation data (Methyl JHU-USC HumanMethylation450) in tumor sample of 97 patients with follicular variant of papillary thyroid carcinoma from TCGA database (<http://tcga-data.nci.nih.gov/>) (April 2016).

Identification of differentially expressed miRNAs and mRNAs

Differentially expressed miRNAs and mRNAs were evaluated in the r-bioconductor package DESeq (21). The Limma package in R was applied to calculate P values by two-tailed Student's *t*-test. MetaMA package in R was utilized to combine P values, and the false discovery rate was obtained from multiple comparisons using the Benjamini and Hochberg method (22). Those differentially expressed miRNAs and mRNAs were identified with the criterion of $P < 0.05$.

Correlation analysis of differentially expressed miRNAs and mRNAs

The pairwise Pearson correlation coefficients between differentially expressed miRNAs and mRNAs were calculated, and $P < 0.05$ was considered as statistical significance. Six miRNA-target prediction tools (miRWalk, miRanda, miRDB, RNA22, PICTAR2 and Targetscan) were used 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. We selected the miRNA-target pairs with negative correlations to establish the miRNA-target regulatory network, which was visualized using Cytoscape software (23).

Functional analysis of target mRNAs of differentially expressed miRNAs

In order to understand the biological function of target

Table 1 Eight differentially expressed miRNAs in follicular variant of papillary thyroid carcinoma

miRNA	log2Fold change	P value
Up-regulated miRNAs		
hsa-mir-221	3.544169565	2.80E-04
hsa-mir-222	3.21622386	8.37E-04
hsa-mir-34a	2.666407907	9.17E-03
hsa-mir-1251	2.072575193	4.00E-02
Down-regulated miRNAs		
hsa-mir-138-2	-0.982954891	4.47E-02
hsa-mir-675	-2.176635768	4.56E-02
hsa-mir-138-1	-0.972380058	4.79E-02
hsa-mir-214	-1.350097616	4.96E-02

mRNAs of differentially expressed miRNAs, we conducted Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis based on the online software GENECODIS (24). FDR <0.05 was set as the criterion for selecting significantly enriched GO and KEGG terms of target differentially expressed mRNAs.

Influence of DNA methylation on differentially expressed mRNAs

The COHCAP package in R (<https://sourceforge.net/projects/cohcap/>) (25) was used to screen the differential methylation sites between tumors and normal tissues that are more likely to regulate downstream gene expression. Methyl.cutoff =0.5, unmethyl.cutoff =0.3, delta.beta.cutoff =0.1 and false discovery rate <0.05 were considered as differentially methylated CpG sites. Based on the mRNA expression of the follicular variant of papillary thyroid carcinoma, we identified the aberrant DNA methylated CpG sites which affected corresponding mRNA expression.

QRT-PCR in vitro

Five patients diagnosed with follicular variant of papillary thyroid carcinoma and five normal individuals were enrolled in this study. Both the tumor and corresponding normal tissue samples were resected at the time of surgery, and immediately frozen in liquid nitrogen. All participating individuals provided informed consent with the approval of the ethics committee of General Hospital of Jinan Military

Region of PLA. In addition, our study conforms to the provision and in accordance with the Helsinki Declaration.

Total RNA of the tissue samples was extracted using the TRIzol[®] Reagent (TIANGEN) according to the manufacturer's protocols. Two µg RNA was applied to synthesize DNA by SuperScript[®] III Reverse Transcriptase (TIANGEN). Then real-time PCR was performed in an ABI 7300 real-time PCR system with SYBR[®] Green PCR Master Mix (Applied Biosystems). All reactions were carried out in triplicate. Relative gene expressions were analyzed by $2^{-\Delta\Delta Ct}$ method.

Results

MiRNA and mRNA expression pattern

In this study, a total of 97 tissue samples were included in the present study, all of which with fully characterized miRNA profiles, mRNA profiles and DNA methylation data. There were 8 differentially expressed (4 up-regulated and 4 down-regulated) miRNAs and 973 differentially expressed (554 up-regulated and 419 down-regulated) mRNAs with $P < 0.05$ between the follicular variant of papillary thyroid carcinomas and normal tissue. Eight identified differentially expressed miRNAs and top 10 up- and down-regulated differentially expressed mRNAs were listed in *Table 1* and *Table 2*, respectively. The heat map of all differentially expressed miRNAs and top 50 differentially expressed mRNAs was showed in *Figure 1* and *Figure 2*, respectively.

Correlations of differentially expressed miRNAs and mRNAs

In order to investigate the correlations between differentially expressed miRNAs and mRNAs, the correlation analysis was performed. Depending on the correlation analysis, 881 miRNA-mRNA pairs which were negatively correlated ($P < 0.05$, $r < -0.2$) were identified. In the targeted analysis, 466 miRNA-mRNA pairs including 218 miRNA (up-regulation)-mRNA (down-regulated) pairs and 248 miRNA (down-regulation)-mRNA (up-regulated) pairs were screened out. The established regulatory network of miRNA-targeted mRNA with negative correlation was showed in *Figure 3*. The network was consisted of 6 differentially expressed (3 up-regulated and 3 down-regulated) miRNAs and 65 differentially expressed (25 up-regulated and 40 down-regulated) mRNAs. In

Table 2 Top 10 up- and down-regulated differentially expressed mRNAs in follicular variant of papillary thyroid carcinoma

Gene ID	Symbol	log2Fold change	P value
Up-regulated mRNAs			
401498	TMEM215	9.972515	0.000169
170261	ZCCHC12	8.728899	0.004099
6585	SLIT1	8.139089	0.000174
222171	PRR15	7.043451	0.000195
200879	LIPH	6.588005	3.60E-05
55220	KLHDC8A	6.559447	1.10E-06
2561	GABRB2	6.261426	0.013721
10686	CLDN16	5.924016	0.026352
120892	LRRK2	5.57023	2.91E-05
51440	HPCAL4	5.501627	0.032491
Down-regulated mRNAs			
6366	CCL21	-5.06223	0.000305
90632	C6orf176	-4.21365	0.018373
221091	LRRN4CL	-4.12688	1.69E-05
5649	RELN	-4.02531	6.50E-09
347	APOD	-3.94221	2.28E-07
1805	DPT	-3.83603	3.31E-06
57528	KCTD16	-3.81913	4.87E-06
130497	OSR1	-3.81534	0.000239
125	ADH1B	-3.5074	0.001008
10894	LYVE1	-3.48926	4.08E-07

addition, there were 71 nodes and 92 edges in the network. Interestingly, hsa-mir-222 (degree =33), hsa-mir-221 (degree =29), hsa-mir-214 (degree =13), hsa-mir-138-2 (degree =11) and hsa-mir-34a (degree =4) were miRNAs that regulated the most target mRNAs.

Functional analysis of target differentially expressed mRNAs of differentially expressed miRNAs

To understand the potential function of target differentially expressed mRNAs of differentially expressed miRNAs, we conducted GO and KEGG pathway analysis. The results demonstrated that these targeted differentially expressed mRNAs were most significantly enriched in the biological processes of signal transduction, angiogenesis and cell adhesion. Additionally, focal adhesion, pathways in cancer, gap junction, cytokine-cytokine receptor interaction, the MAPK signaling pathway, p53 signaling pathway and ECM-receptor interaction were the significantly enriched signaling pathways of targeted differentially expressed mRNAs. Top 15 significantly enriched GO terms (biological process) and KEGG terms of targeted differentially expressed mRNAs were listed in *Table 3* and *Table 4*, respectively.

Influence of DNA methylation on differentially expressed mRNAs

Generally, the methylation of CpG islands in the promoter regions results in the mRNA silencing. There were 186 differentially methylated CpG sites with FDR <0.05, which

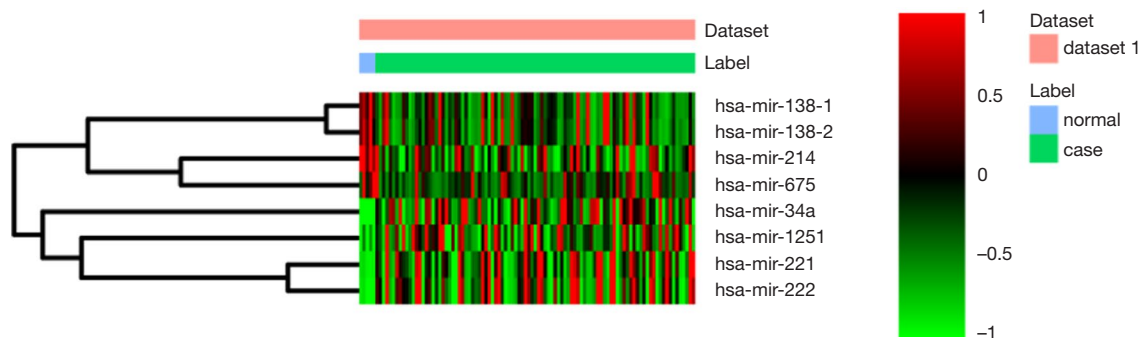


Figure 1 The heat map of all differentially expressed miRNAs in the follicular variant of papillary thyroid carcinoma. Diagram presents the result of a two-way hierarchical clustering of all differentially expressed miRNAs and samples. The clustering is constructed using the complete-linkage method together with the Euclidean distance. Each row represents a differentially expressed miRNA and each column, a sample. The differentially expressed miRNA clustering tree is shown on the right. The colour scale illustrates the relative level of differentially expressed miRNA expression: red, below the reference channel; green, higher than the reference.

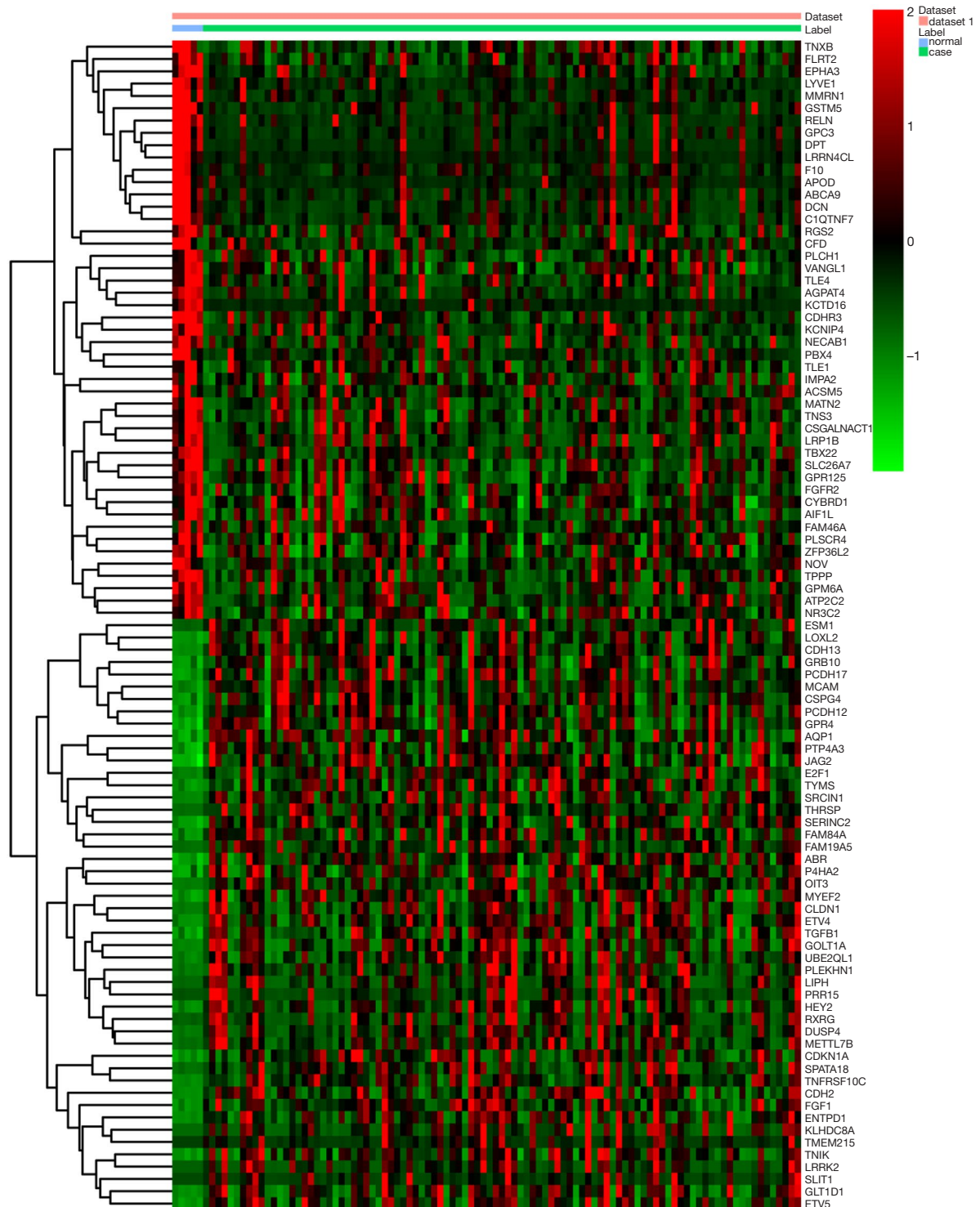


Figure 2 The heat map of the top fifty differentially expressed mRNAs in the follicular variant of papillary thyroid carcinoma. Diagram presents the result of a two-way hierarchical clustering of the top fifty differentially expressed mRNAs and samples. The clustering is constructed using the complete-linkage method together with the Euclidean distance. Each row represents a differentially expressed mRNA and each column, a sample. The differentially expressed mRNA clustering tree is shown on the right. The colour scale illustrates the relative level of differentially expressed mRNA expression: red, below the reference channel; green, higher than the reference.

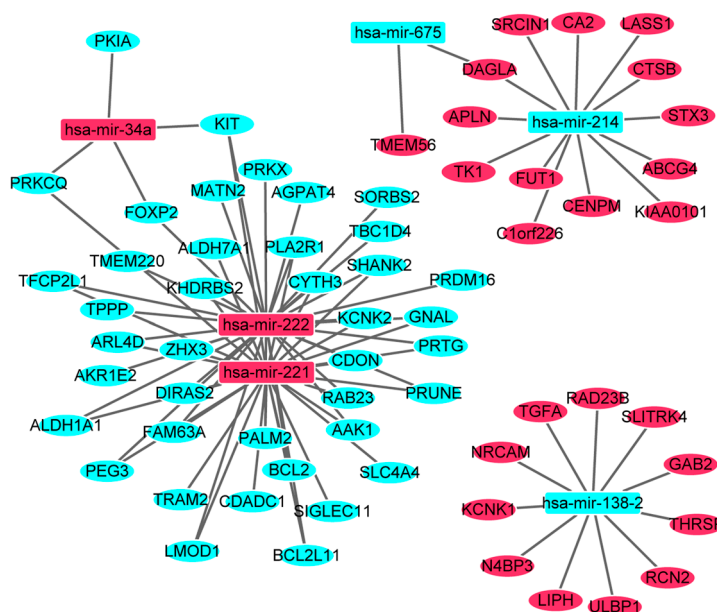


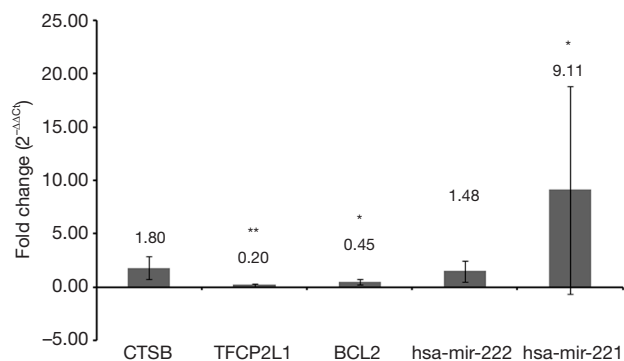
Figure 3 The network of miRNA-target mRNAs with negative correlation between 6 differentially expressed miRNAs and 65 differentially expressed mRNAs in the follicular variant of papillary thyroid carcinoma. The rectangle and ellipses represent the differentially expressed miRNAs and target differentially expressed mRNAs, respectively. The red and blue colors represent up-regulation and down-regulation, respectively.

Table 3 The top 15 significantly enriched biological process of target differentially expressed mRNAs

GO ID	GO term	Number of genes	False discovery rate
0007165	Signal transduction	42	1.09E-10
0001525	Angiogenesis	16	8.12E-10
0007155	Cell adhesion	27	9.64E-10
0030168	Platelet activation	17	1.42E-08
0007275	Multicellular organismal development	33	1.65E-08
0030324	Lung development	10	1.79E-07
0007596	Blood coagulation	15	2.10E-07
0001701	In utero embryonic development	6	3.30E-07
0042493	Response to drug	4	3.62E-07
0032026	Response to magnesium ion	4	3.62E-07
0032355	Response to estradiol stimulus	4	3.62E-07
0010544	Negative regulation of platelet activation	4	3.62E-07
0001666	Response to hypoxia	6	1.17E-06
0030335	Positive regulation of cell migration	4	1.24E-06
0032355	Response to estradiol stimulus	9	5.20E-06

Table 4 The top 15 significantly enriched signaling pathways of target differentially expressed mRNAs

KEGG ID	KEGG items	Number of genes	False discovery rate
Hsa04510	Focal adhesion	15	1.01E-07
Hsa04810	Regulation of actin cytoskeleton	13	3.69E-06
Hsa05215	Prostate cancer	7	3.72E-06
Hsa05200	Pathways in cancer	16	4.37E-06
Hsa05218	Melanoma	6	6.60E-06
Hsa05214	Glioma	6	3.66E-05
Hsa04360	Axon guidance	9	4.27E-05
Hsa04540	Gap junction	4	4.32E-05
Hsa04060	Cytokine-cytokine receptor interaction	12	5.64E-05
Hsa04010	MAPK signaling pathway	3	8.88E-05
Hsa04115	p53 signaling pathway	3	8.88E-05
Hsa04512	ECM-receptor interaction	7	9.65E-05
Hsa04210	Apoptosis	7	9.73E-05
Hsa04020	Calcium signaling pathway	9	1.97E-04
Hsa05221	Acute myeloid leukemia	5	4.81E-04

**Figure 4** Validation differentially expressed miRNAs and mRNAs in the follicular variant of papillary thyroid carcinoma tissues by qRT-PCR. *P<0.05; **P<0.01.

involved 146 mRNAs. However, all these differentially methylated mRNAs were not differentially expressed mRNAs identified above. Therefore, we mainly focused on the study of differentially expressed miRNAs and mRNAs in the follicular variant of papillary thyroid carcinoma.

qRT-PCR

Five pairs of follicular variants of papillary thyroid

carcinoma and adjacent normal tissues were used to validate the expression of identified differentially expressed miRNAs and mRNAs. Based on the above analysis, 2 differentially expressed miRNAs (hsa-mir-222 and hsa-mir-221) and 3 differentially expressed mRNAs (CTSB, TFPC2L1 and BCL2) were randomly selected for validation (Figure 4). The qRT-PCR results demonstrated that hsa-mir-221 was significantly up-regulated (P<0.05), TFPC2L1 and BCL2 were remarkably down-regulated (P<0.01 and P<0.05, respectively). Hsa-mir-222 and CTSB were up-regulated without significance. All these results were consistent data with the integrated analysis.

Discussion

Identification of changes of miRNAs and mRNAs in different levels occurring in tumor development is an important step in fighting against the follicular variant of papillary thyroid carcinoma. In this study, we integrated miRNA expression, mRNA expression data and DNA methylation profiles of follicular variant of papillary thyroid carcinoma to find valuable miRNAs and mRNAs which were highly associated with the follicular variant of papillary thyroid carcinoma. In the network of

miRNA-mRNA with the negative correlation, we obtained 5 differentially expressed miRNAs including hsa-mir-222 (up-regulation), hsa-mir-221 (up-regulation), hsa-mir-34a (up-regulation), hsa-mir-214 (down-regulation) and hsa-mir-138-2 (down-regulation) that regulated the most target mRNAs.

Hsa-mir-222 is a cancer-related miRNA and can promote the proliferation of cancer cell (26). It is reported that up-regulated expression of hsa-mir-222 is characteristic of papillary thyroid carcinoma (27). In addition, over expression of hsa-mir-222 is significantly associated with tumor aggression, central lymph node metastases, extrathyroidal invasion and recurrence, which were considered as the independent predictor of papillary thyroid carcinoma prognosis (28-31). Visone *et al.* found that hsa-mir-221 was excessively secreted in papillary thyroid carcinoma (32). Over expression of hsa-mir-221 is remarkably correlated with tumor aggression and clinic-pathological characteristic of papillary thyroid carcinoma (28,29). It has been demonstrated that hsa-mir-221 could be a potential prognostic biomarker for the recurrence in papillary thyroid carcinoma (33). In this study, we found the relationship between hsa-mir-222, hsa-mir-221 and follicular variant of papillary thyroid carcinoma. In addition, we found that 7 down-regulated differentially expressed mRNAs including 3 cell apoptosis related mRNAs (BCL2, BCL2L11 and PEG3), ALDH1A1, PLA2R1, TFPC2L1 and RAB23 were commonly regulated by hsa-mir-222 and hsa-mir-221.

BCL2, apoptosis regulator (BCL2), an antiapoptotic protein, is involved in biological processes of cell survival and blocking of apoptosis (34,35). It is found that the over expression of BCL2 is associated with aggressiveness of papillary thyroid carcinoma (36). BCL2 like 11 (BCL2L11) is a pro-apoptotic gene and involved in controlling cell death. Bim protein, encoded by BCL2L11, has been regarded as functionally relevant in rat follicular thyroid cells (37). Paternally expressed 3 (PEG3) is associated with cell death and apoptosis. PEG3 encodes the tumor suppressor and is down-regulated in several cancer types, such as glioma, gynecologic cancer, ovarian cancer and invasive cervical cancer (38-41). This suggested that cell apoptosis plays an important role in the process of follicular variant of papillary thyroid carcinoma.

It is reported that aldehyde dehydrogenase 1 family member A1 (ALDH1A1) is down-regulated in papillary thyroid carcinoma (42). In addition, the expression of ALDH1A1 is significantly associated with factors (such as

lymph node metastasis and extrathyroidal extension) that lead to poor prognosis of papillary thyroid carcinoma (43). Finn *et al.* found that phospholipase A2 receptor 1 (PLA2R1) was down-regulated in malignant papillary thyroid carcinoma, and appears to suppress tumorigenesis by activating the tyrosine kinase JAK2 (44). It is noted that PLA2R1 is associated with the prognosis of papillary thyroid carcinoma (45). Kim *et al.* found that lower expression of TFPC2L1 was down-regulated in papillary thyroid carcinoma compared to normal thyroid sample (46). It is worth mentioning that the expression of TFPC2L1 is detected in the follicular variant of papillary thyroid carcinoma (47). RAB23, member RAS oncogene family (RAB23) is a protein that belongs to the Rab family of GTPases. It has been demonstrated that over expression of RAB23 promotes proliferation, migration, invasion and metastasis of tumor cell (48,49). It is noted that the expression of RAB23 is decreased in the follicular variant of papillary thyroid carcinoma compared to the benign follicular adenoma (50).

It is reported that hsa-mir-34a is up-regulated in tissues and cell lines of papillary thyroid carcinoma (51,52). Moreover, the expression level of hsa-mir-34a is associated with tumor invasion and showed the potential diagnostic value of papillary thyroid carcinoma (53). Herein, we found up-regulated expression of hsa-mir-34a in the follicular variant of papillary thyroid carcinoma. Furthermore, protein kinase C theta (PRKCC) and prune exopolyphosphatase (PRUNE) were two of targets of hsa-mir-34a. PRKCC is related to immune response and down-regulated in the brain metastatic of papillary thyroid carcinoma (54). It is found that the expression of PRUNE is detected in anaplastic thyroid cancer and promotes invasion, migration and metastasis of anaplastic thyroid cancer cell (55).

Hsa-mir-214 is significantly down-regulated in tissues and cells of papillary thyroid carcinoma compared with normal, which was remarkably associated with tumor size, TNM stage and lymph node metastasis. In this study, we found that the expression of hsa-mir-214 is down-regulated in the follicular variant of papillary thyroid carcinoma. Moreover, Thymidine kinase 1 (TK1) was one of targets of hsa-mir-214. TK1 is involved in DNA repair and internationally recognized as the marker of abnormal cell proliferation. It is noted that up-regulated expression of TK1 is closely associated with active tumor growth (56). Furthermore, serological TK1 could be an important marker for the early risk of malignancy development (57).

It has been demonstrated that hsa-mir-138-2 has a diagnostic value for papillary thyroid carcinoma (53). In the present study, we found that the expression of hsa-mir-138-2 was decreased in the follicular variant of papillary thyroid carcinoma. In addition, cathepsin (CTSB) was regulated by hsa-mir-138-2. CTSB, a lysosome enzyme, is over expressed in tumor endothelial and epithelial cells. It is found that CTSB is significantly up-regulated in the tumor tissues of thyroid cancer.

According to the KEGG analysis, we found that focal adhesion, MAPK signaling pathway and p53 signaling pathway were three significantly enriched signaling pathways of targeted differentially expressed mRNAs of differentially expressed miRNAs. The focal adhesion signaling pathway is important in maintaining cellular physiology. It is reported that the focal adhesion signaling pathway play a crucial role in the pathogenesis of renal cell carcinoma (58). It is found that constitutive activation of MAPK signaling pathway plays an important role in thyroid carcinoma tumorigenesis (59). Moreover, MAPK signaling pathway is a promising therapeutic target for thyroid cancer (60-62). P53, the tumor suppressor, regulates a lot of biological processes including DNA damage, cellular senescence and apoptosis (63,64). It is reported that p53 mutation is recently detected in about 40% of papillary thyroid carcinoma (65).

Conclusions

In summary, our integrated analysis of the TCGA data led to a number of differentially expressed miRNAs, mRNAs and methylated mRNAs. The epigenetic modifications via five miRNAs (hsa-mir-222, hsa-mir-221, hsa-mir-34a, hsa-mir-214 and hsa-mir-138-2) for BCL2, BCL2L11 and PEG3, ALDH1A1, PLA2R1, TFCEP2L1, RAB23, TK1 and CTSB may be involved in tumorigenesis of follicular variant of papillary thyroid carcinoma. The study of epigenetic alterations between these miRNAs and mRNAs is of value to investigate the pathogenesis of follicular variant of papillary thyroid carcinoma. However, there are limitations to our study. Firstly, the sample in the QRT-PCR is small and larger numbers of tumor tissues are needed for validation; secondly, the deeper mechanism study of the disease such as the animal model and cell culture are also needed.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.06.30>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All participating individuals provided informed consent with the approval of the ethics committee of General Hospital of Jinan Military Region of PLA. In addition, our study conforms to the provision and in accordance with the Helsinki Declaration.

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