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BRAF V600E mutation and microRNAs are helpful in distinguishing papillary thyroid malignant lesions: Tissues and fine needle aspiration cytology cases

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ABSTRACT

Aims: Mutations of BRAF oncogene are considered to contribute in the invasiveness and poor clinicopathologic features of papillary thyroid cancer (PTC). As a step towards understanding the underlying molecular mechanisms of this contribution, we aimed to examine the association of four microRNAs' (miR-222, -137, -214, -181b) levels with BRAFV600E and clinicopathological features in PTC tissues and fine needle aspiration (FNA) specimens.

Methods: In total, 56 PTC and 27 benign with multinodular goiter tissue samples, 95 FNA samples, and B-CPAP and HEK293 cell lines were examined. BRAFV600E mutation was examined in PTC tissues and FNA samples. Expression of microRNAs was assessed by real-time quantitative reverse transcription-PCR.

Key findings: The frequency of BRAFV600E in PTC tissues and FNA samples "suspicious for PTC" was 41.1 and 36.8%, respectively. MiR-222, -137, -214, and -181b were significantly upregulated in PTC tumors ($P < 0.05$) and in B-CPAP cell line ($P < 0.001$). In FNA, the expressions of miR-222, -181b and -214 were significantly elevated in patients suspected for PTC ($P < 0.05$), while there was no significant difference in miR-137. After adjustment for age and sex, miR-181b was associated with an increased risk of bearing BRAFV600E mutation (OR: 1.27; 95% CI: 1.01–1.61; $P = 0.045$) and risk of lymphovascular invasion (OR: 1.66; 95% CI: 1.01–2.72; $P = 0.045$); miR-137 was associated with the risk of larger tumor size (OR: 1.31; 95% CI: 1.04–1.65; $P = 0.022$); miR-222 was related to increase in extracapsular invasion (OR: 1.28; 95% CI: 1.04–1.57; $P = 0.018$).

Significance: Upregulation of miR-222, -214 and -181b has been confirmed in PTC tumors, FNA samples and cell line. MiR-137 upregulation has been confirmed in PTC tumors and cell line, but not in FNA samples. MiR-222, -137 and -181b showed an association with the degree of malignancy in PTC tumors.

1. Introduction

Papillary thyroid carcinoma (PTC) is the most prevalent endocrine malignancy, accounting for 80–90% of all the subtypes of follicular epithelial cell-derived thyroid cancers. PTC is associated with early mortality, low quality of life, and increased health care expenditure [1]. Although PTC is known as a mild disease with good prognosis, it is accompanied with the risk of the worst clinical outcomes such as lateral lymph node metastasis, massive extrathyroid extension, distant metastasis, and advanced Tumor, Node and Metastasis (TNM) stages [2]. Although there are numerous techniques available to determine metastases or recurrences in patients with PTC (*e.g.* diagnostic whole body scan, neck ultrasonography, and serum thyroglobulin measurements) [3,4], fine-needle aspiration (FNA) represents the gold standard method for evaluating thyroid nodules [5]. Approximately 15–30% of thyroid nodules, not being clearly benign or malignant, are categorized as indeterminate, with 5–75% risk for malignancy [6].

Understanding the molecular mechanisms underlying the pathophysiology of PTC could help in improving the diagnostic accuracy of indeterminate FNA cytology. The B-type raf proto-oncogene (BRAF)

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mutation is one of the most distinguished oncogenic genetic variations, occurring in over 45% of PTC cases [7]. Although mutation in BRAF is recognized to be associated with invasive clinical outcomes and poor clinicopathologic properties [8], the molecular mechanisms underlying its contributions to the development and progression of PTC remain unknown.

MicroRNAs (miRs) are small non-coding RNAs (19–25 nucleotides) that control gene expression at the post-transcriptional levels, by targeting mRNAs. They act as negative regulators in the expression of protein-encoding genes which are involved in major processes such as development, apoptosis, cell proliferation, immune response, and hematopoiesis [9]. It is believed that the aberrant expression of miRs is related to numerous human cancers and metastases. Recent gene expression profiling studies have revealed that the differences in profiles may offer a new tool for distinguishing malignant from benign thyroid lesions [10,11], and thereby, help to avoid unnecessary complications and costs associated with thyroid surgery [12]. Performing molecular analyses, such as somatic mutation testing and miRs expression profiling, enables us to classify the diagnosed patients as low and high risk. Some studies revealed that mutation in the BRAF gene is related to the expression of several miRs. In a Chinese population, it was found that miR-221, -222, -146b and -181 were upregulated in PTC patients with tumors that bear BRAF mutations [13]. In contrast, in a study by Cahill et al. the effect of BRAF mutation on miR-137, -222, and -181b downregulation in BRAF mutated cell lines was observed using microRNA analysis [14].

Although the effect of BRAF mutation on the regulation of miRs has been studied, the documented results are controversial in different populations. Therefore, in this study, we aimed to examine the association of four miRs' (miR-222, -137, -214, -181b) expression levels with BRAF V600E mutation and clinicopathological features in PTC tissues, cell line, and FNA samples in an Iranian population.

2. Materials and methods

2.1. Tissue collection and clinicopathological data

Patients who underwent total thyroidectomy were selected over a one-year period (2015–2016), from Erfan and Atiyeh hospitals (in Tehran, Iran). A pathologist rechecked all samples, and confirmed the diagnoses of PTC subjects and those with benign multinodular goiter, also isolating the non-tumoral tissues from tumoral specimens. In total, 83 cases met the eligibility criteria, including 56 PTC cases (tumoral tissues and the adjacent unaffected thyroid tissues from the same patients) and 27 benign cases (non-tumoral thyroid tissues from patients with multinodular goiter). Samples were collected at the time of thyroidectomy in RNase and DNase free tubes, and stored at −80 °C for further examinations. To obtain the data related to stage of thyroid tumors, the staging system created by the American Joint Committee on Cancer (AJCC) was used [15]. The demographic, clinical and pathological data of all patients were collected.

2.2. FNA sample collection

Ninety-five samples were obtained from patients of the Taleghani Hospital, whose thyroid nodules were confirmed by ultrasonography. These nodules were aspirated preoperatively with a 25-gauge needle, and transferred directly into 300 mL of TRIzol reagent (Invitrogen U.S. Cat. No. 15596-026). Three to 5 needle passes were performed for each thyroid nodule, and samples were used for nucleic acid isolation. A cytopathologist reviewed all FNA specimens, and the Bethesda criteria were adopted for assessing the adequacy of FNA samples. The cytological reports were collected and samples classified according to the Bethesda System for Reporting Thyroid Cytopathology [6]. Overall, 45 samples were categorized as benign, 9 were in the Atypia of undetermined significance/follicular lesion of undetermined significance category, 16 were placed in the non-diagnostic or unsatisfactory category, 6 in the follicular neoplasias category, and 19 were categorized as suspicious for PTC. The cytological diagnosis was received before molecular testing.

All clinical specimens examined in this study, and the protocols were approved by the Ethics Committee of the Research Institute for Endocrine Sciences (RIES), Shahid Beheshti University of Medical Sciences (25ECRIES93/10/23). An informed consent was obtained from all patients. The study was conducted in accordance with the declaration of Helsinki, as well as, the RIES guidelines. The authors declare that the study has been done according to ethical standards.

2.3. Cell culture

Normal human embryonic kidney cells, HEK293 (as control cell line) [16], were donated from Dr. Samira Mohammadi-Yeganeh (http://fa.pasteur.ac.ir/userfiles/file/CellBank/cv97/Tissue%20list.pdf), and human PTC cell line (B-CPAP) was purchased from Pasteur Institute of Iran (http://fa.pasteur.ac.ir/userfiles/file/CellBank/cv97/Tumour%20index.pdf). After preparation, the cells were cultured in Dulbecco's Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin and streptomycin (Life Technologies, Gibco). The cells were cultured in a humidified incubator supplemented with 5% CO_2. For evaluation of miRs' expression, approximately 106 cells/well were plated in 6-well plates. After 24 h, the medium was removed, and the cells were washed and harvested.

2.4. RNA/DNA extraction

Total RNA and genomic DNA were extracted from collected tissue specimens and FNA samples by TRIzol reagent (Invitrogen U.S. Cat. No. 15596-026). The total RNA was extracted from cell lines by AllPrep DNA/RNA/Protein Mini Kit (QIAGEN Germany, Cat. No. 80004), according to the manufacturer's instructions and after histological control. RNA and DNA concentrations of all samples were determined using the NanoDrop ND-1000 spectrophotometer at 260 and 280 nm (Thermo Scientific, Waltham, and Mass).

2.5. BRAF V600E mutation detection

BRAF V600E mutation was identified in the DNA extracted from tissue and FNA samples, by direct sequencing of the 251 bp PCR-amplified products. For sequencing, exon 15 was amplified using primer pairs: 5′-CTT CAT AAT GCT TGC TCT GAT AGG-3′ (Forward) and 5′-TTC TAG TAA CTC AGC AGC ATC TCA-3′ (Reverse).

2.6. MiRNA samples and quantitative real-time PCR

Total RNA (1 μg) was reverse transcribed using the microRNA cDNA synthesis kit (PARSGENOME, Iran), according to the manufacturer's protocol, and stored at −20 °C for later use. Amplification condition for miRs was optimized by quantitative reverse transcriptase real-time PCR, using the Rotor-Gene 6000 instrument (Corbett Research, Sydney, Australia); U6 snRNA was used as internal control to normalize miRs expression levels. Experiments were duplicated for tissue samples and triplicated for cell lines. PCR amplification was performed in 25 μL volumes using SYBR Green master mix (PARSGENOME, Iran). Relative quantitation of miRs' expression was done using the comparative CT method [17].

2.7. Statistical analysis

Normal distribution of data was determined by the one-sample Kolmogorov-Smirnov test. Group comparisons of nominal and ordinal variables were performed using the x^2 test and linear-by-linear

Table 1
The clinicopathological characteristics of PTC patients in BRAF mutation.

Clinicopathological characteristics	Number (%)
PTC patients	56
Age	
< 45 years	41 (73.2)
≥45 years	15 (26.8)
Sex	
Male	14 (25.0)
Female	42 (75.0)
BRAF V600E mutation	23 (41.1)
Tumor size	
< 2 cm	40 (71.4)
≥2 cm	16 (28.6)
TNM staging	
I	45 (80.4)
II	2 (3.6)
III	7 (12.5)
IV	2 (3.6)
Focality status	
Unifocal	11 (19.6)
Multifocality	18 (32.1)
Extracapsular invasion	13 (23.2)
Lymph node metastasis	24 (42.9)
Lymphovascular invasion	7 (12.5)
Variant	
Classic	48 (85.7)
Follicular	7 (12.5)
Oncotic (Hurtle cell)	1 (1.8)

TNM: tumor node metastasis.

association test, respectively; all categorical variables were expressed as frequency and percentage. Normal distributed variables were expressed as mean ± standard deviation. U Mann-Whitney test was used to determine the significance of the miRs' expression levels. An age and sex adjusted logistic regression model was carried out to evaluate the association between the expression of miRs and clinicopathological characteristics in PTC patients. All analyses were performed using the SPSS software (version 20.0 for Windows; SPSS Inc., Chicago, IL); with P value < 0.05 being considered as significant. Graphs were illustrated using the MedCalc software (version 14.8.1). The REST 2009 Software (Qiagen, Hilden, Germany) was applied to assess the expression of miRs in cell lines.

3. Results

Of 27 benign subjects, 22 (81.5%) were female; the mean age of male and female participants was 55.6 ± 7.13 and 46.4 ± 13.9 years, respectively (P > 0.05). Clinicopathological information of 56 PTC patients is summarized in Table 1. From the patients with complete data, 15 (26.8%) were aged ≥45 years, 16 (28.6%) had tumor size ≥2 cm, 9 (16.1%) were classified in higher stages (III/IV), 23 (41.1%) were BRAF V600E positive, and 48 (85.7%) had the classical variant of PTC. Extracapsular invasion, lymph node metastasis, and lymphovascular invasion were presented in 13, 24, and 7 patients, respectively. Of the 95 collected FNA samples, 86 (90.5%) were female; the mean ages of male and females were 50.2 ± 20.6 and 44.9 ± 13.1 years, respectively. BRAF V600E mutation was detected in 44.4% of the Atypia of undetermined significance/follicular lesion of undetermined significance category, 6.3% of non-diagnostic or unsatisfactory category, and 36.8% of the suspicious for PTC category.

3.1. MiRs levels in human PTC tissues, cell lines and FNA samples

The expression of miR-222, -137, -214, and -181b in PTC tumors compared to the benign, and in tumoral tissues compared to the adjacent non-tumoral tissues is presented in Fig. 1A and B. MiR-222, -137, -214 and -181b were significantly upregulated in human PTC cells (B-CPAP) compared to control cell line (HEK293) (P < 0.001) (Fig. 2). In FNA samples, the expressions of miR-222, -181b and -214 were significantly elevated in patients suspected for PTC compared to those with benign lesions (P = 0.010, P = 0.048, and P = 0.046, respectively), while there was no significant difference in miR-137 expression (P = 0.635) (Fig. 3).

3.2. MiRs levels in human PTC tissues and clinicopathological features

The levels of miR-222, -137, -214 and -181b according to patients' age, gender, BRAF V600E mutation status, tumor size, TNM stage, focality status, extracapsular invasion, lymph node metastasis, and lymphovascular invasion are presented in Fig. 4A to I.

3.3. Association of miRs' expression and clinicopathological features

Logistic regression analysis (Table 2) revealed that after adjustment for age and sex, miR-181b expression was associated with an increased risk of bearing BRAF V600E mutation (odds ratio (OR): 1.27; 95% confidence interval (CI): 1.01–1.61; P = 0.045); miR-137 expression was associated with an elevated risk of larger tumor size (OR: 1.31; 95% CI: 1.04–1.65); P = 0.022); miR-222 expression was related to an increase in extracapsular invasion (OR: 1.28; 95% CI: 1.04–1.57; P = 0.018); and miR-181b expression was associated with increased risk of lymphovascular invasion (OR: 1.66; 95% CI: 1.01–2.72; P = 0.045).

4. Discussion

In this study, we examined the association of miR-222, -137, -214 and -181b expression levels with BRAF V600E mutation and clinicopathological features in PTC tissues, cell line, and FNA samples in an Iranian population for the first time. After adjustment for age and sex, logistic regression showed that the odds of miR-181b expression in the BRAF (+) group was about 1.27 times the BRAF (−); also, in those with lymphovascular invasion it was around 1.66 times those without invasion; miR-137 expression in higher tumor size was approximately 1.31 times lower tumor size; miR-222 expression in extracapsular invasion was nearly 1.26 times the group without invasion.

The BRAF V600E mutation, which leads to constitutive activation of the MAPK pathway, is the most common mutation in PTC. Activation of this pathway is a common and important mechanism in the genesis and progression of human carcinomas, due to increasing cell division and proliferation [18]. Recently, studies have mainly focused on FNA samples for finding useful biomarkers to prevent unnecessary surgery; of which, BRAF mutation and miRs' expression levels are the most prominent. Brown et al. investigated the BRAF mutation in 78 FNAs of thyroid lesions, and suggested that testing supernatant DNA in FNA specimens may increase the diagnostic power by 1/11 (9%) [19]. However, Fnais et al. in a systematic review and meta-analysis based on the literature, found no strong evidence to support the implementation of BRAF V600E mutation analysis plus FNA as a single screening test for patients with suspicious and indeterminate thyroid nodules [20]. In a study, Paskas et al. evaluated the diagnostic value of the BRAF V600E mutation, miR-221 and -222 expressions, and galectin-3 protein in FNA samples with indeterminate cytology. With a reliable prediction, they demonstrated a reduction of the initial 120 patients to 58; thus, the number of individuals undergoing surgery decreased by half [21]. Moreover, miRs' signature in thyroid FNA cytology was investigated in Atypia of undetermined significance cases [22].

The association of BRAF V600E mutation with aggressive PTC characteristics has been reported in several studies [23–25]. Previously, we had also reported that this mutation was associated with larger tumor size and lymph node metastasis in the studied population [26]. Although the association of miRs' expression with BRAF V600E mutation and the clinicopathological features of PTC were investigated in

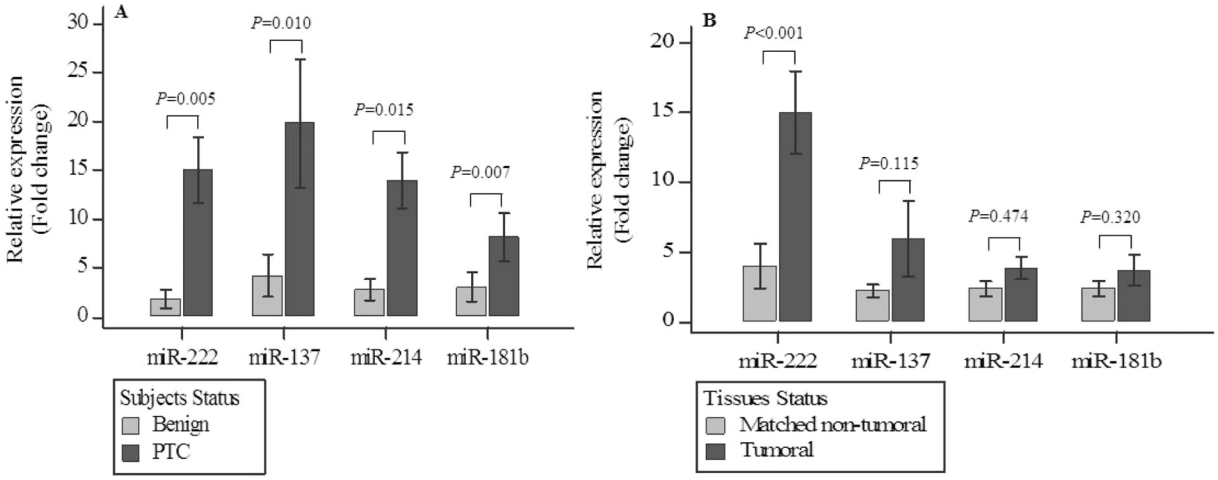

Fig. 1. MiRs' relative expression levels; A) PTC patients *vs*. benign multinodular goiter, B) tumoral tissues *vs*. adjacent non-tumoral tissues of PTC patients.

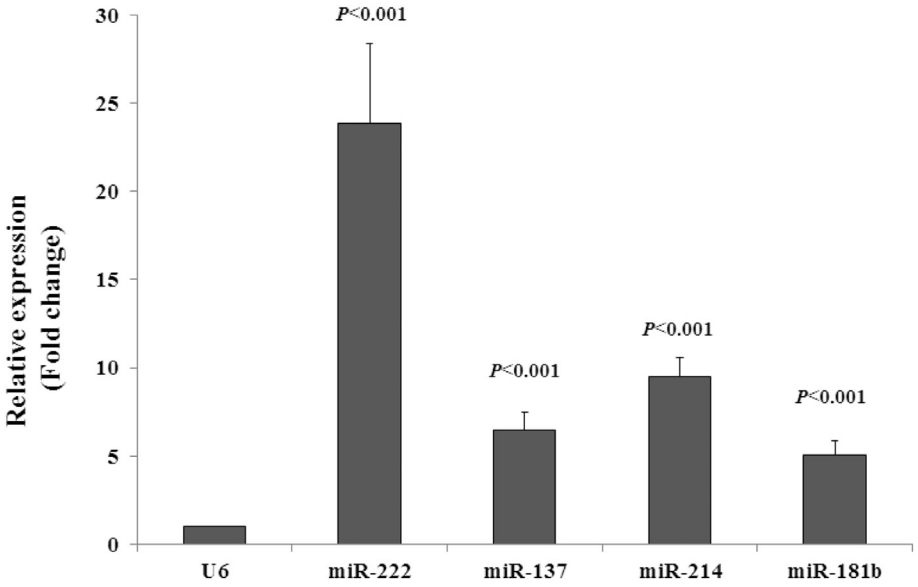

Fig. 2. MiRs' relative expression levels in B-CPAP cell line *vs*. HEK293 cell line.

numerous studies, conflicting results have been reported. Recently, researchers indicated that some miRs are more expressed in severely aggressive forms of PTC, and can be used as biomarkers for the recurrence of this cancer [27–29]. Sun et al. demonstrated that miR-221, -222, 146b and -181 were overexpressed in PTC patients with BRAF mutation [13]. However, Sheu et al. showed no correlation between BRAF V600E mutation and the expression of miR-221, -222, -146b, -181 and -21 in PTC subjects [30]. In the current study, miR-181b expression level was significantly associated with BRAF V600E mutation in PTC patients. Controversies in the reports of these studies could be due to different samples of various origins, and differences in the types of PTC, sample size, techniques and population in these studies. Thus, further investigations are needed to elucidate the interaction between the expression of miRs and BRAF mutation in the pathogenesis and progression of PTC. It seems that BRAF mutation, previously known by its role in the MAPK pathway, plays a distinguished role in the onset of tumorigenesis, which was confirmed in this study. Perhaps the onset of the inhibition process and uncontrolled proliferation may lead to variations in the expression of these miRs.

It has been revealed that miR-222, which is encoded in tandem on the X chromosome, is upregulated in numerous human cancers, including PTC [31,32]. A list of genes have been reported as potential targets for miR-222; of which, the p27/kip1 and p57/kip2 genes are cell cycle inhibitors and tumor suppressors downregulated *via* overexpression of miR-222 in cancer cell lines [33]. Mardente et al. suggested that abnormal expression of miR-222, induced by HMGB1 might interfere with PTEN regulation of the cell cycle [34]. In cancers, HMGB1, is related to invasion and metastasis *via* the RAGE pathway, including activation of NFκB, MAPKs, PI3K/Akt, Rho GTPases, JAK/STAT and Src family kinases. PTEN, as a tumor suppressor gene, is involved in the RAGE transduction network and is a potential target for miR-222 [35]. Tetzlaff et al. showed the upregulation of miR-222 in some cases of multinodular goiter compared to PTC [36], suggesting that overexpression of miR-222 may be an early event in thyroid carcinogenesis. In the current study, after adjustment for age and sex, a significant association was observed between miR-222 levels and tumors with extracapsular invasion in PTC patients. Sun et al. found an association between miR-222 overexpression and BRAF (+) and lymph node metastasis [13]; however, in contrast, they reported that upregulation of miR-222 was significantly associated with higher TNM stage; whereas, we found no such association in our study. Moreover, Chou et al. demonstrated that the expression level of miR-222 was significantly associated with extrathyroidal invasion [37]. Overall, upregulation of miR-222 may be involved in the development, invasion

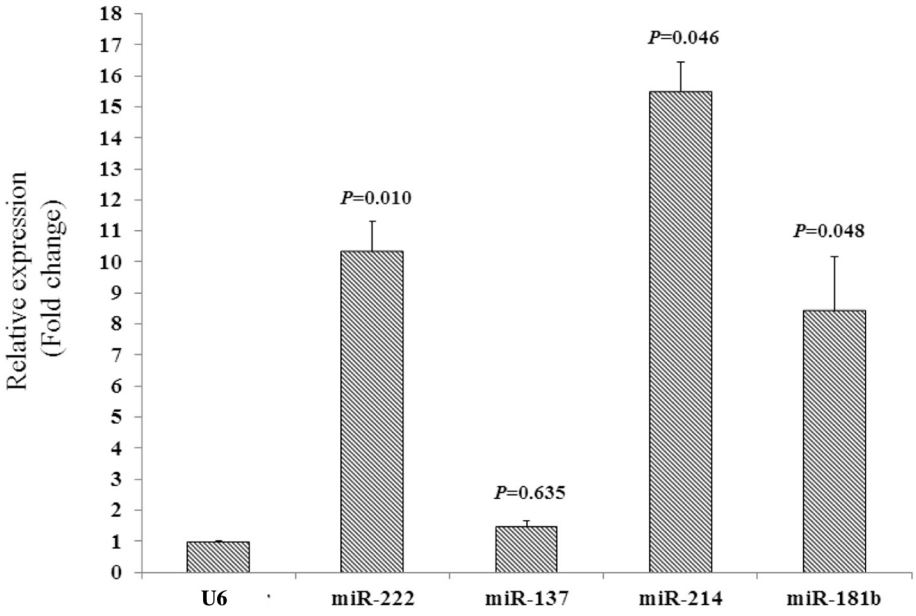

Fig. 3. MiRs' relative expression levels in FNA PTC suspicious samples *vs.* benign ones.

and metastasis of PTC.

MiR-137 has a tumor suppressor role in many human cancers [38,39]. Dong et al. reported that miR-137 expression decreased in PTC tissues [40]; they showed that downregulation of miR-137 inhibited cell proliferation, colony formation, and reduced cell migration and invasion ability by targeting CXCL12 in PTC. Moreover, using microRNA analysis, Cahill et al. indicated that miR-137 was downregulated in PTC cell lines with BRAF mutations [14]. In contrast, Xiu et al. reported that miR-137 was upregulated in bladder cancer tissues and cell lines, being considered as an oncomiR. Overexpression of miR-137 promoted cell proliferation, migration and invasion of bladder cancer cells by targeting PAQR3, a tumor suppressor gene [41]. This investigation suggested a controversial role for miR-137 expression, depending on the specific tumor type. In our study, although the expression of miR-137 was not significantly different in PTC suspicious FNA samples compared to the benign, results revealed that it was significantly overexpressed in PTC cell line. After adjustment for age and sex, it was approximately 1.31 times higher in larger tumor size. These differences could depend on the various types of PTC, which in our study, the classic variant was the most frequent. However, the association of miR-137 expression with clinicopathological features, and the functions underlying the molecular mechanism remain unclear, requiring more investigations.

MiR-214 overexpression is involved in the regulation of normal and cancer cell biology, and is associated with human malignancies; however, it can function in a contradictory manner in various carcinomas [42–44]. Zhu et al. suggested that overexpression of miR-214 promotes the progression of osteosarcoma by targeting the Wnt/β-catenin signaling pathway, and indicated that miR-214 is an oncogene for human osteosarcoma [45]. In another study, Wang et al. demonstrated that miR-214 acts as a potential oncogene in breast cancer by targeting the PTEN-PI3K/Akt signaling pathway [46]. The role and underlying molecular mechanism of miR-214 in PTC is unknown. However, Nikiforova et al. detected an upregulation of miR-137 and -214 in anaplastic thyroid carcinoma (ATC), in 60 surgically thyroid neoplastic and non-neoplastic specimens [47]. Recently, Peng et al. showed an overexpression of the miR-200a-3p/miR-214-3p cluster in PTC tumors *versus* benign tissues using ArrayStar analysis [48]. In our study, we found that miR-214 was significantly upregulated in FNA specimens and PTC cell line. After adjustment for age and sex, it was not associated with any clinicopathological features. However, it seems that in later tumorigenesis stages, increased miR-214 expression elevated the tendency for invasion, progression to higher stages of disease and angiogenesis. Changes in the expression of miRs are effective in the formation of new tumoral foci. Although miR-214 expression has no effect on extracapsular invasion, it facilitates the development of metastasis.

MiR-181b activity has been evaluated in cancers other than PTC [49]. It was demonstrated that miR-181b acts both as an oncogene and tumor suppressor, depending on the origin of tissues and cells. An elevated level of miR-181b has been observed in some human tumors [50,51]; while reduced levels of this miR has also been seen in other human carcinomas [52,53]. Meng et al. reported that miR-181 may be involved in the differentiation and invasion of hepatocellular cancer stem cells, by targeting RASSFIA, TIMP3, and NLK genes [54]; miR-181b can promote cell-cycle progression through the downregulation of CBX7, a member of the polycomb repressive complex 1 [55]. The upregulation of miR-181b has been also studied in PTC [36]. Li et al. found that downregulation of miR-181b causes cellular growth inhibition and promotes cellular apoptosis by targeting CYLD in PTC [56]. In the current study, miR-181b was overexpressed in FNA cases and in PTC cell line. After adjustment for age and sex, it was associated with BRAF mutation and lymphovascular invasion, suggesting that this miR plays an oncogenic role in PTC. Consistent with our findings, Sun et al. showed that miR-181 expression was higher in PTC patients compared to those with benign tumors, while also being higher in PTC patients with larger tumor size [13]. Consequently, it can be suggested that upregulation of miR-181b leads to malignant cell transformation in PTC.

The association of miRs with clinicopathological features and FNA samples suggests that these miRs may serve as diagnostic biomarkers prior to surgical procedures for PTC patients. Results showed that these four miRs could be used as possible tools to differentiate subjects with PTC from those with benign multinodular goiter. However, distinguishing between tumoral and adjacent non-tumoral tissues in PTC patients, using these miRs (except for miR-222) is difficult, because not all cells that undergo early changes necessarily start the tumorigenesis process, and only a few will receive subsequent mutations and ultimately create a tumor mass. Elevation of these miRs indicates that they may increase malignancy by targeting some tumor suppressor genes; finding those genes could helpful in the prediction and even treatment of the disease.

To the best of our knowledge, for the first time, the current study

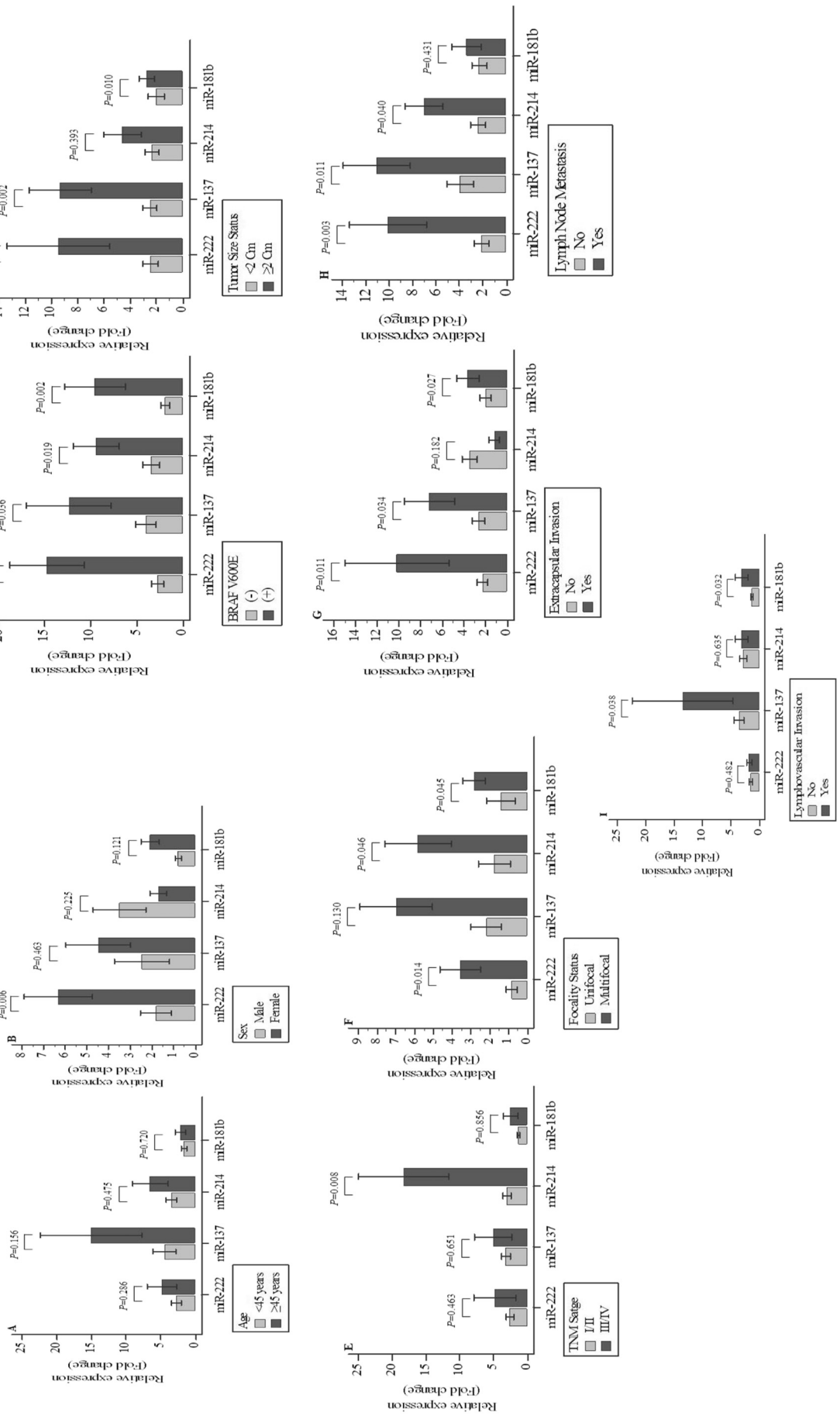

Fig. 4. MiRs' relative expression in PTC patients' specimens with different clinicopathological features; A) patients with age < 45 *vs.* ≥ 45 years, B) males *vs.* females, C) patients with BRAF V600E (−) *vs.* BRAF V600E (+), D) patients with tumor size < 2 cm *vs.* ≥ 2 cm, E) patients with TNM stages I and II *vs.* III and IV, F) patients with unifocal tumors *vs.* multifocal tumors, G) patients without extracapsular invasion *vs.* with extracapsular invasion, H) patients without lymph node metastasis *vs.* with lymph node metastasis, I) patients without lymphovascular invasion *vs.* with lymphovascular invasion.

Table 2
Adjusted logistic regression of the association between clinicopathological risk factors and miRs expression in PTC patients.

Clinicopathological features	MiRNAs	B	P value	Adjusted OR	95% CI for EXP(B)	
					Lower	Upper
Patients[a]	miR-222	0.31	**0.020**	1.36	1.04	1.76
	miR-137	0.00	0.987	1.00	0.97	1.03
	miR-214	0.11	0.215	1.12	0.93	1.34
	miR-181b	0.11	0.283	1.12	0.91	1.38
Tissues[b]	miR-222	0.08	**0.013**	1.08	1.02	1.16
	miR-137	0.15	0.083	1.17	0.98	1.39
	miR-214	0.05	0.458	1.05	0.92	1.20
	miR-181b	0.04	0.499	1.04	0.93	1.16
BRAF V600E	miR-222	0.19	0.068	1.21	0.99	1.49
	miR-137	0.08	0.248	1.09	0.94	1.25
	miR-214	0.17	0.064	1.18	0.99	1.42
	miR-181b	0.24	**0.045**	1.27	1.01	1.61
TNM stages	miR-222	0.07	0.887	1.07	0.42	2.72
	miR-137	−0.52	0.228	0.59	0.25	1.39
	miR-214	0.42	0.092	1.52	0.93	2.48
	miR-181b	1.07	0.301	2.93	0.38	22.49
Tumor size	miR-222	0.10	0.270	1.11	0.92	1.33
	miR-137	0.27	**0.022**	1.31	1.04	1.65
	miR-214	0.09	0.410	1.10	0.88	1.37
	miR-181b	−0.03	0.862	0.97	0.71	1.33
Extracapsular invasion	miR-222	0.25	**0.018**	1.28	1.04	1.57
	miR-137	0.21	0.057	1.24	0.99	1.54
	miR-214	−0.81	0.212	0.44	0.12	1.59
	miR-181b	0.02	0.884	1.02	0.78	1.33
Lymph node metastasis	miR-222	0.24	0.071	1.27	0.98	1.64
	miR-137	0.14	0.087	1.16	0.98	1.36
	miR-214	0.18	0.109	1.19	0.96	1.48
	miR-181b	0.05	0.547	1.05	0.89	1.23
Lymphovascular invasion	miR-222	0.30	0.429	1.34	0.65	2.80
	miR-137	0.08	0.087	1.08	0.99	1.19
	miR-214	0.09	0.502	1.10	0.84	1.44
	miR-181b	0.51	**0.045**	1.66	1.01	2.72

Bold values denote statistical significance at the $p < 0.05$ level.
[a] PTC *vs.* benign.
[b] Tumoral tissues *vs.* adjacent non-tumoral tissues.

evaluated the expression of some miRs, including miR-222, −137, −214 and -181b, in PTC patients and FNA samples from an Iranian population. Moreover, this is the first report on the associations between miR-214 and -137 expressions with clinicopathological characteristics in PTC samples. However, limitations of the present study were small sample size, some missing information on certain clinicopathological features of the patients which could have an effect on the results, and lack of available clinicopathological information for FNA samples after surgery.

In conclusion, the upregulation of miR-222, -214 and -181b has been confirmed in PTC tumors, FNA samples and cell line. Upregulation of miR-137 has been confirmed in PTC tumors and cell line, but not in FNA samples. Among these miRs, miR-222, -137 and -181b showed an association with malignancy in PTC tumors. Considering the role of these miRs in carcinogenesis, identifying their target genes in PTC tumor is suggested.

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Authors' contribution

Study concept and design: Zarkesh & Hedayati; acquisition of data: Fanaei; analysis and interpretation of data: Zarkesh & Nozhat; drafting of the manuscript: Zarkesh & Zadeh-Vakili; critical revision of the manuscript for important intellectual content: Azizi & Hedayati; statistical analysis: Akbarzadeh; study supervision: Hedayati & Zadeh-Vakili.

Conflict of interest

The authors declare that they have no relevant conflicts of interest.

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