Serum miRNAs as Biomarkers for the Diagnosis and Prognosis of Thyroid Cancer: A Comprehensive Review of the Literature

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Keywords

miRNA · Biomarker · Thyroid cancer · Fine-needle aspiration biopsy

Abstract

Background/Objectives: Thyroid cancer is the most common endocrine malignancy and accounts for 1% of cancers. In recent years, there has been much interest in the feasibility of using miRNAs or miRNA panels as biomarkers for the diagnosis of thyroid cancer. miRNAs are noncoding RNAs with 21–23 nucleotides that are highly conserved during evolution. They have been proposed as regulators of gene expression, apoptosis, cancer, and cell growth and differentiation. **Methods:** The Directory of Open Access Journals (DOAJ), Google Scholar, PubMed (NLM), LISTA (EBSCO), and Web of Science were searched. **Results:** The serum level of miRNAs (miRNA-375, 34a, 145b, 221, 222, 155, Let-7, 181b) can be used as molecular markers for the diagnosis and prognosis of thyroid cancer in the serum samples of patients with thyroid glands. **Conclusions:** Given that most common methods for the screening of thyroid cancer cannot detect

the disease in its early stages, identifying miRNAs that are released in the bloodstream during the gradual progression of the disease is considered a key method in the early diagnosis of thyroid cancers.

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Introduction

Thyroid cancer is the most common endocrine malignancy and accounts for about 1% of cancers [1, 2]. According to research findings, the degree of malignancy of this cancer in women is 3 times more than that in men, which is probably due to the development and proliferation of estrogen receptors in the cancerogenesis process. The incidence rate of this disease reaches its maximum in the third and fourth decades of life [3]. The high heterogeneous nature of thyroid tumors in terms of characteristics, including pathological-clinical and morphological, along with the lack of specific markers, makes it difficult to differentiate the diverse nature of tumor glands from nontumor ones. Therefore, many efforts have recently

been made to introduce a suitable marker that can predict the diverse nature of thyroid glands and as a diagnostic and prognostic factor alongside other pathological-clinical methods. Approaches to detect thyroid cancer glands include blood tests, imaging tests such as thyroid scan, ultrasound, and fine-needle aspiration biopsy (FNA) [4]. In most cases, imaging tests are not able to differentiate thyroid glands. Also, it should be noted that FNA, which is the best way to diagnose thyroid glands, is not able to differentiate adenomatous follicular tumor glands from carcinomatous follicular glands, and the results of this method are considered suspicious [4, 5]. It is difficult to accurately diagnose thyroid cancer from other benign thyroid conditions such as follicular adenomas, cysts, and goiter. A total of 75% of patients who undergo hemithyroidectomy for the benign disease do not need to do so. In addition, the measurement of thyroglobulin as a non-invasive method cannot be applied to measure long-term surveillance for 25% of patients due to the presence of thyroglobulin antibody. In FNA-aspirated cells, about 30% of thyroid gland biopsies remain undecided or undiagnosed [6]. Tumors of molecular markers such as miRNA are generated by cancer cells or other cells in response to the cancer and are responsible for cells survival processes. At present, an increase or decrease in such tumors can be used in various fields such as screening, diagnosis, and prognosis. These molecular markers are produced in the blood, urine, or body tissues and can be identified using molecular laboratory tests [7].

miRNAs

miRNAs are a group of small noncoding RNAs with a length of 21–23 nucleotides that interact exclusively with their proprietary mRNA, thereby destroying mRNA and inhibiting translation. miRNA genes account for approximately 1% of the genome in different species and have hundreds of target genes in each of them. Over 2,500 miRNAs have been identified in human genomes that regulate 30% of genes encoding proteins. These small regulatory molecules were first identified in 1993. Most of them are located in chromosome fragile sites and are prone to chromosomal removal, movement, and epigenetic changes in various diseases, including cancer. miRNAs target multiple genes at the same time so that the target genes can sometimes reach more than 100 [8, 9]. The relationship between miRNA dysregulation and cancer was identified in 2002 [10].

miRNA Biogenesis

miRNA is transcribed in the nucleus of the gene and produces pri-miRNA. Then, it produces a precursor called pre-miRNA by nuclear RNase III Drosha (endonuclease). The pre-miRNA is transported to the cytoplasm by the Exportin 5 protein. This molecule is broken in the cytoplasm by another enzyme called Dicer and produces a 21–23 double-stranded nucleotide sequence. One of the strands is decomposed and the other is placed in the silencing complex (RNA-RISC). This active complex targets the mRNA in question and binds to the end of mRNA 3'-UTR and exerts its inhibitory effect (Fig. 1). miRNA exerts its effect in regulating gene expression through the inhibition of protein translation or decomposition of mRNA [11–13].

miRNA and Cancer

Small ribonucleic acids (miRNAs) and their expression pattern in cancer provide a new and broad argument in oncology. Recent studies show that miRNAs are impaired in cancer and play an essential role in the development and progression of cancer [14–18]. Therefore, the search for such molecules as a target for the diagnosis, treatment, or prognosis of cancer can offer a promising approach [19, 20]. It is believed that the identification of these miRNAs can be used as cancer prognosis and treatment strategies; for example, the MRX34 drug is an miRNA mimic that is under investigation in phase I clinical trials and is used in the treatment of cancer [21]. Many miRNAs that have been identified to date play a role in cancer treatment. Comparing tumor tissues with normal tissues suggests that miRNAs are placed in the fragile sites of the human genome and may be increased or removed during chromosomal rearrangement. In addition, epigenetic mechanisms may lead to inappropriate expression of miRNA genes and consequently abnormal miRNA expression in tumor tissues that can result in enormous changes in regulating the expression of target mRNA. Some miRNAs have an oncogenic role in the cancer phenotype, and often the defective regulation of these miRNAs can be seen in a wide range of cancers [22]. Oncogenic miRNAs include miRNA-155 and miRNA-21 [23], and members of Let-7 and miRNA-34 families are examples of suppressive miRNAs. Different miRNAs affect different phases of cancer; for example, miRNA-10b regulates metastasis and displays high expression levels in advanced malignancies. The inhibition of miRNA-10b

Fig. 1. miRNA biogenesis: miRNA biogenesis takes place in the nucleus and cytoplasm. Pri-miRNAs are the transcription product of POLII RNA. It is first converted into pre-miRNA beginning in the nucleus by Drosha and later transported to the cytoplasm by

the Exportin 5 enzyme. The miRNA is finally processed by Dicer in the cytoplasm and is converted into RNA-RISC complex, and thus the mRNA that is complementary to this complex is processed through entering its structure [13].

can prevent a new cancer metastasis. The expression of miRNA-335 can inhibit metastasis but cannot block tumor cell proliferation and has no effect on the cell apoptosis rate. However, there may be a number of miRNAs that can inhibit the proliferation of tumor cells and metastasis [24]. Changes in miRNA expression levels, including increased and decreased expression, have been reported in various human cancers [25, 26].

miRNAs and Thyroid Cancer

There is much interest in the feasibility of miRNAs or miRNA panels as biomarkers for the diagnosis of thyroid cancer in recent years. Recent studies have shown impaired miRNA expression in thyroid cancer and its essential role in the development and progression of the disease. In previous studies, miRNA in FNA samples were studied, and finally a number of miRNAs were considered effective in the pathogenesis of thyroid cancer; however, these studies reported inconsistent results [4].

miRNA expression measurement has the potential to be used as an alternative for thyroglobulin measurement in thyroid cancer. Circulating miRNAs (plasma and serum) are released by infected cells, which escape from being destroyed. Currently, there are ongoing research studies on miRNAs as biomarkers for diagnosis, potential cancer recurrence, evaluation of survival, and cancer grading, as well as markers to monitor the pathological progress.

miRNA-221–222

The expression level of miRNA-221–222 is constantly increased in thyroid cancer tissue compared with normal tissue; this increased expression is associated with increased risk of cancer metastases outside the thyroid, lymph node metastasis, distant tissues metastasis, and tumor recurrence [27]. Preliminary studies suggest that serum levels of these miRNAs in thyroid cancer patients are higher than those in healthy controls. The simultaneous use of 221–222 miRNA expression level measurement

and cytological evaluation can enhance the sensitivity and specificity of the FNA test. Homologous 221 and 222 miRNAs are very similar to each other and are located as a cluster on chromosome X. They increase proliferation, telomere activity, angiogenesis, and autophagy and are involved in apoptosis and cell cycle regulation. Targeting this subset of miRNAs can be useful in thyroid papillary carcinoma diagnosis and treatment management [28].

miRNA-375

Two separate studies examined the expression status of miRNA-375 in FNA samples and showed that increased expression of miRNA-375 inhibits papillary cancer cell proliferation and induces apoptosis in cell lines. Studies on animal models and cell lines showed that an increased level of this miRNA decreased migration and the invasion of cancer cells. The ERBB2 molecule is activated when Ras-MAPK and PI3K-AKT pathways are activated, and increased expression of this molecule enhances cell proliferation and reduces apoptosis. ERBB2 is targeted by miRNA-375, and when it is decomposed by this miRNA the proliferation is inhibited; therefore, this miRNA may play a tumor-suppressing role in thyroid cancer in which its expression is decreased [29, 30].

miRNA-155

Increased expression of miRNA-155 in thyroid cancer cell lines and in vitro animal models increases tumor growth. The results suggest that these miRNAs have an oncogenic role. miRNAs target APC, whose reduced expression leads to an increase in cell growth. This miRNA activates the β -catenin pathway, which in turn activates downstream pathways such as C-MYC and TCF. It seems that this miRNA has the potential to be used in the diagnosis of thyroid cancer. This requires further study [31].

miRNA-146b

Studies on the activity and performance of miRNA-146b in cell lines (TPC1 and BCPAP) of papillary thyroid cancer show that when this miRNA is inhibited, migration and invasion are significantly reduced in cell lines; when its expression is increased, migration and invasion are increased as well. The expression level of this miRNA was increased and reduced by mimicking it and anti-

miRNA [32]. SMAD4 is a key protein in the signaling pathway that targets and decomposes TGF- β (an inhibitor of cell migration) [33]. A cell cycle regulatory protein named P27 (kip1) inhibits cell cycle progression in the G1 phase through interaction with cyclins; there is a negative regulatory effect between this protein and the expression levels of miRNA-146b [34]. In tumors that have BRAFT1799A mutation, increased expression of this miRNA leads to a frequent incidence of invasion to surrounding tissues in thyroid cancer. There is a positive correlation between overexpression of this miRNA and invasive thyroid cancer. This miRNA is a positive regulator of cell migration and invasion in thyroid cancer [35, 36]. Currently, common prognostic factors for tumor aggressiveness include tumor size, lymph node metastasis, thyroid-out invasion, and, more recently identified, BRAFT1799A mutation. Measuring the expression of miRNA-146b can be a good alternative to these invasion measurement methods in thyroid cancer.

miRNA-34a

Increased expression of miRNA-34a in thyroid cancer cells leads to increased activity of the PI3K/AKT/Bad pathway. In fact, it has an antiapoptotic effect, and its target gene is GAS1 protein that plays the role of a tumor-suppressing gene. This oncogenic miRNA increases cell proliferation and inhibits apoptosis in thyroid cancer [37]. Computer bioinformatics in the Cancer Genome Atlas Database has shown that miRNA-34a is overexpressed in papillary thyroid cancer [38].

miRNA-Let-7

The results on the role of Let-7 show that it reduces Ras level and serves as a tumor suppressor. Transfecting Let-7 in the TPC-1 cell line that has an RET mutation leads to inhibition of the MAPK pathway, prevents cell proliferation, and increases P21. This is the first report that shows the presence of this miRNA in thyroid cancer. Since Let-7 is a tumor-suppressing miRNA, its expression is likely to decrease in this cancer [39]. The Rs712 sequence is located on the KRAS gene 3'-UTR mutant, which is targeted by Let-7. It has been confirmed that polymorphisms in this region is associated with the risk of developing thyroid cancer [40, 41]. Rs10877887 polymorphism, which is located in the promoter region of Let-7, may be at risk of developing thyroid cancer, and rs13293512

Table 1. Deregulation of miRNA expression in thyroid cancer

miRNA	Target genes	Functional activity	Ref.
miRNAs-221–222	Cell cycle regulation and apoptosis genes	It increases cell proliferation, telomere activity, angiogenesis, and autophagy	28
miRNA-375	ERBB2	Overexpression of this miRNA increases cell proliferation and reduces apoptosis	29, 30
miRNA-155	APC	It activates the β -catenin pathway, which in turn leads to activation of downstream pathways such as C-MYC and TCF	31
miRNA-146b	SMAD4, P27	When this miRNA is inhibited, migration and invasion are significantly reduced in cell lines, and migration and invasion are increased by increasing its expression	32–36
miRNA-34a	GAS1	It increases the activity of the PI3K/AKT Bad pathway and has an antiapoptotic effect	37, 38
miRNA-34a	KRAS	It inhibits the MAPK pathway, prevents cell proliferation, and increases P21 expression level	39–42
Let-7	CYLD	Decreased expression of this miRNA leads to an increase in cell proliferation and inhibition of apoptosis in thyroid cancer cells	43

polymorphism, which is located in the promoter region of Let-7, may be linked with lymph node metastasis in thyroid cancer [41]. In a study, Let-7 expression was measured in the serum of 106 patients with thyroid cancer and was compared with that of 95 patients with benign glands and 45 patients without thyroid problems. It was concluded that the expression of Let-7 serum levels in thyroid cancer was significantly different compared with that in benign glands without thyroid problems [42].

miRNA-181b

The results on the role of miRNA-181b in FNA samples show that reducing its expression leads to inhibition of cell proliferation and an increase in apoptosis in thyroid cancer cells. Its target gene is CYLD, and decreased expression of this miRNA is applicable in the diagnosis and treatment of thyroid cancer [43].

Discussion

By the late 1970s, several blood tests were identified and discovered for different types of cancer antigens. Since then, other markers have also been identified that were not investigated further due to lack of advantages over the current markers. However, none of these mark-

ers secured the main searching goal of the marker, which is to diagnose the cancer in the early stages. Most of the patients had small amounts of these markers in their blood, which makes it difficult to diagnose the cancer in the early stages. The level of these markers exceeds the normal level at late stages of cancer. However, these markers do not increase in all patients with cancer. Considering the reasons mentioned above, the main objective, which was to detect cancer in early phases, has not yet been realized. Current techniques for the diagnosis of thyroid cancer often involve tissue biopsy and FNA. The ideal biomarker is easily accessible, and sampling must be conducted to access such a biomarker conveniently. Since FNA and biopsy techniques are inconvenient for the patient, some studies have been done on biomarkers in body fluids. The purpose of research on molecular tumor markers such as miRNA is to diagnose different cancers using a blood test. This simple blood test not only diagnoses the cancer in early phases, but also prevents many deaths each year. Altogether, according to previous studies, it can be argued that miRNAs can be used to identify people at risk of progression of the tumor. Furthermore, it is very practical to use them for the early diagnosis of cancer, because they can be measured in samples in which a small percentage of tissue is damaged as well as in samples that are in the early phases of the disease. Therefore, miRNAs are considered suitable tumor markers. Prognostic miRNAs can be measured both in serum level and

tumor tissue. In addition, tumor mass or hidden metastases can be diagnosed by examining the expression levels of serum markers. In this regard, the most widely used tissue markers are molecules capable of showing tumor development through increasing cell proliferation or tissue metastasis. One of the ultimate goals of miRNA in thyroid cancer is to develop an expression pattern through which the patient's condition, response to therapy, and risk of metastases can be achieved using circulating miRNAs. Will miRNAs, as diagnostic markers, replace all current methods? More clinical studies are needed. Recent studies show that impaired miRNA regulation is an important step in the development of most cancers. Considering that most common methods for the screening of thyroid cancer are not able to diagnose the disease in the early stages, identifying tumor miRNAs that are released in the bloodstream during the gradual progress of the disease is a key method of diagnosing cancer early.

Conclusion

Studies have shown that miRNAs are impaired in cancer and play an essential role in the development and progression of cancer. Therefore, searching for miRNA as a target for the prognosis or diagnosis of thyroid cancer can provide a promising approach. Given that most common

methods of screening thyroid cancer are not able to diagnose the disease in the early stages, identifying tumor miRNAs that are released in the bloodstream during the gradual progression of the disease is a key method in the early diagnosis of cancer. As a result, miRNAs can be used as molecular markers in the diagnosis and prognosis of thyroid cancer in serum samples and as diagnostic and prognostic biomarkers in combination with other diagnostic-clinical methods by measuring serum levels in the near future. By determining and comparing the serum levels of miRNAs (miRNA-375, 34a, 145b, 221, 222, 155, Let-7, 181b) in the serum samples of patients with thyroid glands, the diverse nature of thyroid glands can be predicted (Table 1). These miRNAs can also be used as biomarkers to examine cancer recurrence, survival rate, and cancer grading, as well as invasion in thyroid cancer.

Acknowledgment

This work was supported by a grant from Shahrekord University of Medical Sciences, Shahrekord, Iran (grant No. 1395-01-75-2871).

Disclosure Statement

The authors declare that there are no conflicts of interest.

References

- Scopa CD: Histopathology of thyroid tumors. An overview. *Hormones (Athens)* 2004;3: 100–110.
- Larijani B, Mohagheghi M, Bastanagh M, Mosavi-Jarrahi A, Haghpanah V, Tavangar S, et al: Primary thyroid malignancies in Tehran, Iran. *Med Princ Pract* 2005;14:396–400.
- Holmes L, Hossain J, Opara F: Pediatric thyroid carcinoma incidence and temporal trends in the USA (1973–2007): race or shifting diagnostic paradigm? *ISRN Oncol* 2012;2012:906197.
- Hamburger JI: Diagnosis of thyroid glands by fine needle biopsy: use and abuse. *J Clin Endocrinol Metab* 1994;79:335–339.
- Hoseinpourfeizi MA, Ghanbarian M, Khalaj-Kondori M, Shiri S, Montazeri V, Halimi M: Study of expression pattern of eIF4E gene as a molecular marker in thyroid cancer. *Sci J Kurdistan Univ Med Sci* 2011;16:16–26.
- Graham MER, Hart RD, Douglas S, Makki FM, Pinto D, Butler AL, et al: Serum microRNA profiling to distinguish papillary thyroid cancer from benign thyroid masses. *J Otolaryngol Head Neck Surg* 2015;44:1.
- Mazzaferri EL: Thyroid cancer – changing patterns of diagnosis and treatment. *Business briefing. US Endocrine* 2005;2:1–8.
- Heneghan HM, Miller N, Kerin MJ: miRNAs as biomarkers and therapeutic targets in cancer. *Curr Opin Pharmacol* 2010;10:543–550.
- Slabý O, Svoboda M, Fabian P, Svoboda M, Garajová I, Šachlová M, et al: Association of miR-21, miR-31, miR-143, miR-145 and let-7a-1 levels with histopathologic features of colorectal cancer. *Eur J Cancer* 2007;5(suppl 4):78–79.
- Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al: Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc Natl Acad Sci USA* 2002;99: 15524–15529.
- Bhatt K, Mi Q-S, Dong Z: MicroRNAs in kidneys: biogenesis, regulation, and pathophysiological roles. *Am J Physiol Renal Physiol* 2011;300:F602–F610.
- Bartel DP: MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215–233.
- Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004;116: 281–297.
- Chen C-Z, Li L, Lodish HF, Bartel DP: MicroRNAs modulate hematopoietic lineage differentiation. *Science* 2004;303:83–86.
- Cuellar TL, McManus MT: MicroRNAs and endocrine biology. *J Endocrinol* 2005;187: 327–332.
- Harfe BD, McManus MT, Mansfield JH, Hornstein E, Tabin CJ: The RNaseIII enzyme Dicer is required for morphogenesis but not patterning of the vertebrate limb. *Proc Natl Acad Sci USA* 2005;102:10898–10903.
- Lim LP, Lau NC, Garrett-Engle P, Grimson A, Schelter JM, Castle J, et al: Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* 2005;433:769–773.
- Wilfred BR, Wang W-X, Nelson PT: Energizing miRNA research: a review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. *Mol Genet Metab* 2007;91: 209–217.

- 19 Rossi L, Bonmassar E, Faraoni I: Modification of miR gene expression pattern in human colon cancer cells following exposure to 5-fluorouracil in vitro. *Pharmacol Res* 2007;56:248–253.
- 20 Svoboda M, Izakoviceva Holla L, Seif R, Vrtkova I, Kocakova I, Tichy B, et al: MicroRNAs miR125b and miR137 are frequently upregulated in response to capecitabine chemoradiotherapy of rectal cancer. *Int J Oncol* 2008;33:541.
- 21 Hydbring P, Badalian-Very G: Clinical applications of microRNAs. Version 3. *F1000Res* 2013;2:136.
- 22 Orellana EA, Kasinski AL: MicroRNAs in cancer: a historical perspective on the path from discovery to therapy. *Cancers* 2015;7:1388–1405.
- 23 Medina PP, Nolde M, Slack FJ: OncomiR addiction in an in vivo model of micro RNA-21-induced pre-B-cell lymphoma. *Nature* 2010;467:86–90.
- 24 Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, et al: The 21-nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. *Nature* 2000;403:901–906.
- 25 Xie Q, Chen X, Lu F, Zhang T, Hao M, Wang Y, et al: Aberrant expression of microRNA 155 may accelerate cell proliferation by targeting sex-determining region Y box 6 in hepatocellular carcinoma. *Cancer* 2012;118:2431–2442.
- 26 Zhang Y, Wei W, Cheng N, Wang K, Li B, Jiang X, et al: Hepatitis C virus-induced up-regulation of microRNA-155 promotes hepatocarcinogenesis by activating Wnt signaling. *Hepatology* 2012;56:1631–1640.
- 27 Lee JC, Zhao JT, Clifton-Bligh RJ, Gill A, Gundara JS, Ip JC, et al: MicroRNA-222 and MicroRNA-146b are tissue and circulating biomarkers of recurrent papillary thyroid cancer. *Cancer* 2013;119:4358–4365.
- 28 Zhang R, Hardin H, Chen J, Guo Z, Lloyd RV: Non-coding RNAs in thyroid cancer. *Endocr Pathol* 2016;27:12–20.
- 29 Wang X-Z, Hang Y-K, Liu J-B, Hou Y-Q, Wang N, Wang M-J: Over-expression of microRNA-375 inhibits papillary thyroid carcinoma cell proliferation and induces cell apoptosis by targeting ERBB2. *J Pharmacol Sci* 2016;130:78–84.
- 30 Rossi ED, Bizzarro T, Martini M, Capodimonti S, Sarti D, Cenci T, et al: The evaluation of miRNAs on thyroid FNAC: the promising role of miR-375 in follicular neoplasms. *Endocrine* 2016;54:723–732.
- 31 Zhang X, Li M, Zuo K, Li D, Ye M, Ding L, et al: Upregulated miR-155 in papillary thyroid carcinoma promotes tumor growth by targeting APC and activating Wnt/ β -catenin signaling. *J Clin Endocrinol Metab* 2013;98:E1305–E1313.
- 32 Lima CR, Geraldo MV, Fuziwara CS, Kimura ET, Santos MF: miRNA-146b-5p upregulates migration and invasion of different papillary thyroid carcinoma cells. *BMC Cancer* 2016;16:1.
- 33 Geraldo M, Yamashita A, Kimura E: MicroRNA miR-146b-5p regulates signal transduction of TGF- β by repressing SMAD4 in thyroid cancer. *Oncogene* 2012;31:1910–1922.
- 34 Acibucu F, Dökmetaş H, Tutar Y, Elagoz Ş, Kilicli F: Correlations between the expression levels of micro-RNA146b, 221, 222 and p27Kip1 protein mRNA and the clinicopathologic parameters in papillary thyroid cancers. *Exp Clin Endocrinol Diabetes* 2014;122:137–143.
- 35 Chou C-K, Chen R-F, Chou F-F, Chang H-W, Chen Y-J, Lee Y-F, et al: miR-146b is highly expressed in adult papillary thyroid carcinomas with high risk features including extra-thyroidal invasion and the BRAFV600E mutation. *Thyroid* 2010;20:489–494.
- 36 Yip L, Kelly L, Shuai Y, Armstrong MJ, Nikiforov YE, Carty SE, et al: MicroRNA signature distinguishes the degree of aggressiveness of papillary thyroid carcinoma. *Ann Surg Oncol* 2011;18:2035–2041.
- 37 Ma Y, Qin H, Cui Y: MiR-34a targets GAS1 to promote cell proliferation and inhibit apoptosis in papillary thyroid carcinoma via PI3K/Akt/Bad pathway. *Biochem Biophys Res Commun* 2013;441:958–963.
- 38 Cong D, He M, Chen S, Liu X, Liu X, Sun H: Expression profiles of pivotal microRNAs and targets in thyroid papillary carcinoma: an analysis of The Cancer Genome Atlas. *Onco Targets Ther* 2015;8:2271–2277.
- 39 Marques JC, Fuziwara CS, Yamashita AS, Rezende E, da-Silva MJ, Kimura ET: Effects of let-7 microRNA on cell growth and differentiation of papillary thyroid cancer. *Transl Oncol* 2009;2:236–241.
- 40 Jin H, Liang Y, Wang X, Zhu J, Sun R, Chen P, et al: Association between a functional polymorphism rs712 within let-7-binding site and risk of papillary thyroid cancer. *Med Oncol* 2014;31:1–5.
- 41 Wang Y, Wei T, Xiong J, Chen P, Wang X, Zhang L, et al: Association between genetic polymorphisms in the promoter regions of let-7 and risk of papillary thyroid carcinoma: a case-control study. *Medicine* 2015;94:e1879.
- 42 Yu S, Liu Y, Wang J, Guo Z, Zhang Q, Yu F, et al: Circulating microRNA profiles as potential biomarkers for diagnosis of papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2012;97:2084–2092.
- 43 Li D, Jian W, Wei C, Song H, Gu Y, Luo Y, et al: Down-regulation of miR-181b promotes apoptosis by targeting CYLD in thyroid papillary cancer. *Int J Clin Exp Pathol* 2014;7:7672–7680.