



Expression of miR-127, miR-154, and miR-183 in Medullary Thyroid Carcinoma Tumors

Mahsa RAHMANI SAMANI ^{1,2}, Marjan ZARIF-YEGANEH ², Atefeh MEHRABI ^{1,2}, Amir Nader EMAMI RAZAVI ³, *Sara SHEIKHOLESLAMI ², *Mehdi HEDAYATI ²

1. School of Advanced Sciences and Technology, Tebran Medical Sciences, Islamic Azad University, Tebran, Iran
2. Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences and Metabolism, Shahid Beheshti University of Medical Sciences, Tebran, Iran
3. Iran National Tumor Bank, Cancer Research Center, Cancer Institute of Iran, Tebran University of Medical Sciences, Tebran, Iran

*Corresponding Authors: Email: sara.sheikholeslami@gmail.com, Hedayati@endocrine.ac.ir

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Abstract

Background: Medullary thyroid cancer (MTC) accounts for 5%–10% of all thyroid cancers, but causes 13% of all thyroid cancer related deaths. MicroRNAs (miRs) have key functions in the development and progression of MTC. Altered expression of some miRs has been reported in many human cancers, including Thyroid cancer. Therefore, we aimed to analyze the expression of miR-154, miR-183 and miR-127 in MTC tumor tissues.

Methods: In this case-control study, 15 MTC Formalin-fixed, paraffin-embedded (FFPE) tissue samples and 15 adjacent normal thyroid FFPE tissues, as a control group, were collected from Taleghani, and Loghman Hakim Hospitals, Tehran, Iran since 2005 till 2015. After RNA extraction and cDNA synthesis, the expression of miR-127, miR-154 and miR-183 was measured by quantitative Real-Time Polymerase Chain Reaction (qRT-PCR).

Results: Our data showed a significant increase in the expression of miR-127 in MTC samples in comparison with the control group ($P < 0.05$). Although miR-154 and miR-183 expression levels had increase expression in MTC tumors, this change was not statistically significant.

Conclusion: The miR-127 could be considered as a prognostic, diagnostic and therapeutic marker for the management of MTC, and it is proposed for further investigation to fully establish the role of this miRNA in MTC.

Keywords: Medullary thyroid carcinoma; Gene expression; miR-127; miR-154; miR-183

Introduction

Thyroid cancer is the most frequent endocrine malignancy that affects 12.2 cases per 100,000 people per year in the United States (1). Thyroid tumors are generally divided into four histological types, including papillary carcinoma (PTC), follicular carcinoma (FTC), anaplastic carcinoma (ATC), and medullary carcinoma (MTC) (2). MTC is a neuroendocrine malignancy that arises

from parafollicular C-cells of the thyroid gland (3). Although this type of thyroid cancer comprises approximately 5%–10% of all thyroid cancers, it causes 13% of all thyroid cancer-associated deaths in the world (4). MTCs could generally occur as either sporadic or hereditary forms. Sporadic forms of the disease constitute nearly 75% of MTC cases, while hereditary tu-



mors are related to multiple endocrine neoplasias (MEN) 2 A and B syndromes and familial MTC (FMTC) that affect only 25% of cases (5,6).

Understanding the molecular and genetic abnormalities of these tumors is a good way to find key targets for developing new and effective diagnoses and therapies for MTCs. RET proto-oncogene mutations have been established in MTC pathogenesis (7-10). Genetically, multiple genes and their protein products such as RET, RAS, growth factors and their receptors have been reported to function aberrantly in MTC cells (11). However, epigenetic abnormalities are also involved in neoplastic growth along with genetic alterations. Epigenetic modifications on genes and their products include DNA methylation, histone modifications, nucleosome positioning and non-coding RNAs, especially microRNA expression (12).

MiRNA was first discovered in 1993, and later it was found that miRNAs are highly conserved molecules among various species, function in a tissue-specific manner, and have important roles in biological processes (13). MiRNAs are a class of non-coding RNAs (ncRNAs) with 19–24 nucleotides length that bind to the 3' ends of messenger RNAs, inhibiting the translation of them into protein products. Aberrant function of miRNAs can impair important biological processes leading to the initiation of some diseases such as cancer. These molecules with oncogenic functions are generally called OncomiRs (14). MiRNAs have been identified to influence all of six hallmarks of malignant cells: 1- self-sufficiency in growth signals (let-7 family), 2- insensitivity to anti-growth signals (miR-17-92 cluster), 3- evasion from apoptosis (miR-34a), 4- limitless replicative potential (miR-372/373 cluster), 5- angiogenesis (miR-210), and 6- invasion and metastases (miR-10b) (15). Aberrant expression of miRNAs has been proven to deregulate cell proliferation, differentiation, and apoptosis that cause uncontrolled tumor growth and progression. For example, down-regulation and up-regulation of specific miRNAs may repress tumor suppressor gene and increase oncogene expressions, respectively (16). Plenty of previous studies

have analyzed miRNA expression in various types of thyroid cancer, indicating the dysregulated expression of miRNA in cancer tissues compared to their normal counterparts. Additionally, miRNA expression profiles have been shown to have high variability among the different types of thyroid cancer (17).

However, limited data are available considering the analysis of miRNA profile of MTC tumors and more comprehensive and studies are needed to identify important miRNAs with diagnostic and therapeutic applications (16, 18). Accordingly, we aimed to investigate the expression of miR-154, miR-183 and miR-127 in MTC tumors in comparison to surrounding normal tissues.

Materials and Methods

Human MTC Tissue Samples

In this case-control study, tumor samples were collected from 15 MTC patients and 15 non-tumoral adjacent counterparts. The specimens were obtained from the paraffin-embedded tissue samples of MTC patients' undergone thyroidectomy in the last 10 years since 2005 at Taleghani, and Loghman Hakim Hospitals, Tehran, Iran. Histopathological diagnosis of tumoral and normal areas of all tissues was determined by pathologist using H&E staining.

The Ethical code of this study was IR.SBMU.ENDOCRINE.REC.1395.364 in Research Institute for Endocrine Science, Shahid Beheshti University of Medical Sciences.

RNA extraction, cDNA synthesis and qRT-PCR

Total RNA was extracted from 10µm sections of paraffin-embedded tissues using miRNeasy FFPE Qiagen kit (Cat No.217504. QIAGEN Co. Germany) according to the manufacturer's instructions. Reverse transcription and quantitate Real-Time Polymerase Chain Reaction (qRT-PCR) were completed by the PARSGENOME microRNA RT-PCR system (Tehran, Iran) in three steps as follows: First, PolyA enzyme step was added a polyA tail to 3' end of RNA. Second,

first-strand cDNA synthesis produced specific miR cDNA product using specific primers. Finally, amplification of qRT-PCR with SYBR green master mix and miR specific primers with thermal cycling was done.

After evaluation of RNA purity and integrity, polyadenylation reaction on isolated RNA was performed at 37 °C, and then its concentration was measured by a NanoDrop ND-1000 spectrophotometer (Catalog No.ND2000, Thermo Scientific Co, USA). Thereafter, cDNA molecule was synthesized using 2µl of buffer5x, 1µl of dNTP (10mM), 0.5µl of reverse transcriptase enzyme (RT), 0.5µl of primer for each microRNA (15pmol) and 1µg of Poly-adenylate RNA for 60 min at 44 °C, and then for 1 min at 85 °C (RT deactivation). Finally, the expression levels of miR-127, miR-183 and miR-154 were analyzed by Real-Time PCR using SYBR Green fluorescent dye. U6 was utilized as a reference gene, for data normalization.

To analyze microRNA expression, quantitative reverse transcriptase Real-Time PCR (qRT-PCR) by Rotor-Gene 6000 was used (Corbett Research, Sydney, Australia). Moreover, U6snRNA (RNU6B) was utilized as internal control to normalize the miRNA levels. All reactions were run in duplicate and no template control (NTC) was

involved in each PCR Run. All NTC samples were negative. The $2^{-(\Delta\Delta CT)}$ algorithm was applied to evaluate Relative quantification of miRNA expression

Statistics

The results of this study were analyzed using independent t-test and MedCalc software. The normal distribution of data was evaluated by Shapiro-Wilk test. $P < 0.05$ was considered as a significant level.

Results

Expression levels of miR-127, miR154, and miR-183

Figure 1 shows the alterations observed in the expression of miR-127, miR154 and miR-183 between MTC tumors and control tissues. The expression of miR-127 in thyroid tissues of patients with MTC significantly increased (2.44 times) in comparison to the control group ($P < 0.05$). However, our data revealed no significant increase in the expression levels of miR-183 and miR-154 in MTC tissue samples compared to the control group.

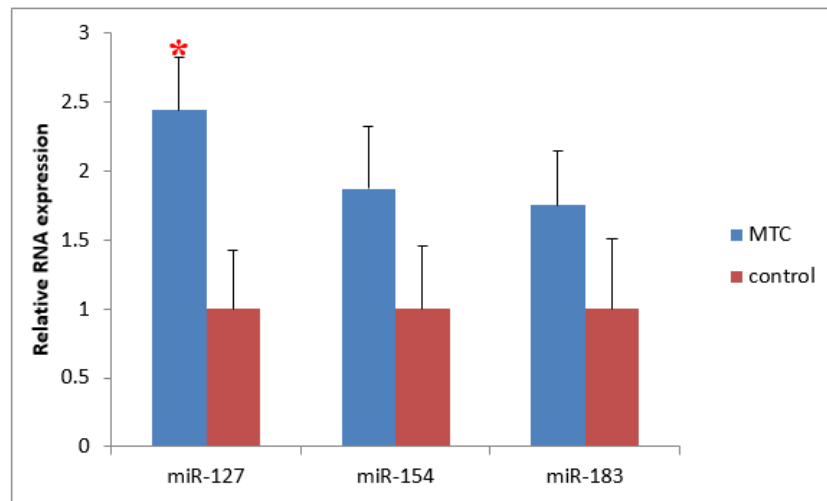


Fig. 1: Comparing the increased expression levels of miR-127, miR154, and miR-183 in MTC and control tissue samples

*Significant increased expression

Discussion

The present study evaluated and compared the expression of miR-127, miR-154 and miR-183 between 15 MTC tumors of the thyroid tissue and their matched control specimens using Real-Time PCR. Our obtained data uncovered an augmented expression level of miR-127 expression in MTC samples compared to the normal tissues. Although miR-154 and miR-183 expression levels had increase expression in MTC tumors, this increase was not significant.

The expression of several miRNAs in surgically removed normal and cancerous thyroid tissue samples was analyzed and found upregulated levels of miR-127, miR-154, and some other miRNAs in MTC specimens (19). Another study analyzed the expression of nine miRNAs using Real-Time PCR method in 34 cases of sporadic MTC, 6 cases of hereditary MTC, and 2 cases of C-cell hyperplasia (CCH). Their results showed a significant increase in all of miRNAs especially our tested miRNAs, miR-127, miR-154 and miR-183, in MTC and CCH samples (20). In another study on miRNA profile of tissue samples from patients with sporadic and hereditary MTC to unravel potential prognostic biomarkers and therapeutic targets in MTC identified an overexpressed miR-183.

The results of that study also clarified the correlation between overexpression of miRs-183 and lateral lymph node metastases, residual disease, distant metastases and mortality. Besides, their *in vitro* studies revealed that knock down of this miRNA could block cell proliferation and upregulation of an autophagy-associated protein called LC3B. Consequently, they suggested an important role for miRNAs in the biology of MTC and acting as prognostic and therapeutic factors (21). Overexpression of mir-127 in our study is following previous works.

In this study, increased levels of miR-154 and miR-183 expression in MTC tumors was not significant. One explanation for these inconsistent results may arise from the type of tissue used to

prepare the tissue specimens. We used paraffin-embedded tissues in this study, but the samples analyzed in the previously mentioned reports were fresh snap-frozen tissues. Micro RNAs are susceptible to various intrinsic and extrinsic factors such as RNA extraction method, individual variances (which include age, race, diet, exercise, medications and chemicals), and the method of tissue preparation and maintenance. Moreover, some variables such as warm ischemia time, fixation, and storage can affect miRNA profiles of fixed tissues (22). The other reason for this inconsistency could result from the type of MTC from which the tissues have been obtained. For example, there are differential expressions of miR-9, miR-183, and miR-375 between sporadic and hereditary MTC tissue samples (21). More interestingly, the expression of miR-127 in sporadic MTCs with somatic RET mutations has been proven to be significantly lower than the cases with wild-type RET (20). MiR-127 has been shown to have a role as a potential prognostic factor for patients with breast cancer and is conversely associated with proliferation and invasiveness of breast cancer cells (23, 24). MiR-183 and mir-154 have also been reported as predicting, prognostic and therapeutic targets in different types of cancers (25, 26). These microRNAs may play their roles in specific stages or grades of a tumor.

Therefore, the lack of information about the stage and grade of tumor, size of the dissected tumor and metastasis were our study limitations. Besides, increasing the sample size may influence the results of future studies on MTC tumors. Various factors could affect the profile of miRNAs in cancers, and hence the inconsistency between our data and previous works may be a result of these differences.

Conclusion

Our data provided evidence to highlight a prognostic, diagnostic, and therapeutic potential for miR-127 in the tumor tissue of MTCs. However,

further studies with larger sample sizes are required to establish the precise role of this mRNA in different aspects of MTC development and spreading. The results of the present study proposed the evaluation of the larger amount of mRNAs with a broader range of roles in this type of cancer to discover key biomarkers of MTC development and progression and aid in the clinical management of these tumors.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interests.

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