Verification of differentially expressed miRNA in parathyroid tumors of sporadic and hereditary forms

YOONJUNG CHUNG

Department of Medical Science

The Graduate School, Yonsei University

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Directed by Professor Yumie Rhee

The Master's Thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

YOONJUNG CHUNG

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This certifies that the Master's Thesis of

Yoonjung Chung is approved.

Thesis Supervisor: Yumie Rhee

Thesis Committee Member #1: Eun Jig Lee

Thesis Committee Member #2: Jae Myun Lee

The Graduate School
Yonsei University

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ABSTRACT

Verification of differentially expressed miRNA in parathyroid tumors of sporadic and hereditary forms

YOONJUNG CHUNG

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BACKGROUD

MicroRNAs (miRNAs) have been shown to be dysregulated in tumors; however, miRNA regulation of parathyroid tumorigenesis remains poorly understood. Here, we report expression of miRNA and correlation with clinical features in hereditary and sporadic parathyroid tumors to better understand the epigenetics changes.

METHODS

miRNA arrays containing 887 human miRNAs were performed on total RNA extracted from parathyroid tumor samples from 6 patients with primary hyperparathyroidism (3 with sporadic tumors and 3 with hereditary tumors in patients with multiple endocrine neoplasia type 1, MEN 1) and 2 normal parathyroid tissues. Differentially expressed miRNA were validated by the TaqMan real-time quantitative PCR using RNU6 as endogenous control in 15

sporadic and 10 hereditary parathyroid tumors. Relative quantification was performed by Livak method with 20 normal parathyroid tissues as a calibrator.

RESULTS

Using FDR < 0.05 from microarray data, we identified 10 differentially expressed miRNAs between normal parathyroid tissues *vs.* those from sporadic or hereditary tumors. Among them, putative tumor-suppressor miR-199b-5p was differentially expressed; significantly decreased and negatively correlated with PTH level in sporadic parathyroid tumors, meanwhile, upregulated and positively correlated with PTH level in hereditary parathyroid tumors. In agreement with these findings, clinical manifestations are much severe in sporadic parathyroid tumors than hereditary tumors regarding high calcium level correlated with a high PTH and larger tumor size. Even though there was not any significant direct relation between Menin target genes with miR-199b-5p, integration pathway analysis revealed a network between Menin itself and miR-199b-5p associated with cell cycle and proliferation.

CONCLUSION

Down-, or up-regulation of miRNA-199b-5p seems to have a distinct role in the tumorigenesis leading to the phenotypic differences in sporadic and hereditary type of parathyroid tumors. This argues for the involvement of miRNAs with different manner in the pathogenesis of parathyroid tumors under different genetic background.

Key words : miRNAs, MEN 1, parathyroid tumors

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YOONJUNG CHUNG

Department of Medical Science
The Graduate School, Yonsei University

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I. INTRODUCTION

Parathyroid tumor is the most frequent causes of primary hyperparathyroidism (PHPT). Majority of parathyroid tumors occurs sporadically in up to 90% of cases, but 5% of them also may be a major component of familial syndromes, as in multiple endocrine neoplasia type 1 (MEN 1) [1]. MEN 1 is an autosomal dominant inherited disorder characterized by the development of tumors mainly in the parathyroid glands, the endocrine pancreas, and the anterior pituitary gland [2-4]. Among these manifestation, PHPT is the most frequently expressed form of MEN 1, occurring in more than 90% of MEN-1 mutation carriers [5].

According to gene array analysis, hereditary parathyroid tumors in MEN 1 patients were clustered with sporadic parathyroid tumors, indicating that these tumors may share a similar genetic pathway of tumorigenesis [6].

However, multiplicity, recurrence, and an earlier age of onset are quite distinct features of the MEN 1-related parathyroid tumors compared to the sporadic tumors [7]. According to the continuum model, partial inactivation of tumor suppressors can contribute to the different course of the tumorigenesis such as in MEN1-related parathyroid tumors [8]. Therefore, one of major epigenetic differences such as microRNAs (miRNAs) might explain some of the different clinical features between two parathyroid tumor types. However, it has not been fully elucidated yet.

miRNAs are small noncoding RNAs, considered as a class of gene products functioning as negative regulators of gene expression [9]. The miR expression patterns have been extensively investigated in many types of tumors, and this pattern may be used to investigate tumor cell biology, classify tumor subtypes, and identify diagnostic and prognostic markers [10]. Recently, differentially expressed miRNAs in parathyroid tumors by analyzing the miRNA profiles in normal and hyperplastic parathyroid tissue as well as benign and malignant parathyroid were reported [11]. No data about the differentially expressed miRNAs in parathyroid tumor of sporadic and hereditary form are currently available. We analyzed whether the different expression of miRNA in sporadic and hereditary parathyroid tumors exists and any clinic-pathological correlation occurs.

II. MATERIALS AND METHODS

1. Patients and parathyroid tissue samples

We obtained 25 parathyroid tumor samples along with clinical and histo-pathological data. Ten hereditary parathyroid tumor tissues were acquired from MEN1 patients who were confirmed to have the germline mutations. Clinical and genotypic information of all hereditary parathyroid tissues are shown in the Table 1. The 20 normal parathyroid gland tissues and 15 sporadic parathyroid adenoma samples were obtained from either thyroidectomy or parathyroidectomy procedure. All the samples (45 parathyroid tissues) were stored at -80℃ deep freezer.

Table 1. Germline mutations and clinical characteristics in the patients with MEN 1

Patient No.	Sex/Age (y)	Germline mutation[a]			Phenotype		
		Exon	c.DNA nomenclature	Protein nomenclature	Pituitary	Pancreas	Others
M1	F/62	2	c.111dupT[†]	p.Ser38Phefs	prolactinoma	neuroendocrine carcinoma gastrin+	gastric neuroendocrine tumor
M2	F/29	2	c.196_200dupAGCCC	p.Ser66fs	prolactinoma	non-functioning	adrenal adenoma,
M3	M/38	2	c.225_226ins T[†]	p.76Yfs	acromegaly	non-functioning	Cushing`s syndrome, adrenal spinal cord ependymoma
M4	F/59	3	c.491C>A	p.Ala164Asp	-	non-functioning	empty sella
M5	M/39	3	c.628_631delACAG	p.Thr210fs	-	neuroendocrine carcinoma	stomach carcinoid tumor
M6	F/81	7	c.1049A>T[†]	p.Asp350Val	prolactinoma	non-functioning	-
M7	F/49	7	c.1049A>T[†]	p. Asp350Val	prolactinoma	non-functioning	leiomyoma in the bladder, the uterus, and the esophagus adrenal adenoma R/O liver neuroendocrine tumor
M8	F/39	Intron 9	c.1350+2T>G	splicing	non-functioning	non-functioning	liver hemangioma and cyst
M9	F/38	Intron 9	c.1350+2T>G	splicing	non-functioning	-	gallbladder polyps
M10	M/48	10	c.1508G>A [†]	p.Gly503Asp	Rathke's remnant	-	gallbladder adenomyomatosis

[a]Mutations are numbered in relation to the *MEN1* cDNA reference sequence (GenBank accession number NM_130799.1).

[†], Novel mutations in Korea.

2. Total RNA extraction and miRNA array

Total RNA extracted from pulverized 45 parathyroid tissue samples using Trizol reagent (GIBCO, BRL, Gaithersburg, MD, USA) according to manufacturer protocol. Following extraction, total RNA were quantified by Nanodrop. Total RNA was stored at -80℃.

RNA labeling and hybridization on RNA microarray chips were performed by Agilent Human miRNA 8 X 15K (Rel 14.0 V2), which contains 887 human miRNAs with four duplicate probes per miRNA. There were total RNA of 2 normal parathyroid tissues, 3 sporadic parathyroid tumor tissues and 3 hereditary parathyroid tumor (MEN 1) tissues.

Hybridization signals were detected by Agilent surescan microarray scanner. Scanner images were analyzed by Agilent feature extraction software. Data normalization was performed by Genowiz 4.0.5.6.

3. cDNA synthesis

A. mRNA cDNA synthesis

To evaluate mRNA expression level of specific gene of interest. High-Capacity cDNA archive kit (Applied Biosystems, Carlsbad, CA, USA) have been used. The genes were *CDC73* (*HRPT2*, parafibromin), *HES1*, *CCND1,* and *LIN7C*. Reverse transcriptase reaction included purified total RNA 2 μg in a final volume 20 $\mu\ell$ following manufacturer protocols.

B. miRNA to reverse transcription synthesis

Reverse transcription synthesis for validating miRNAs performed by Taqman miRNA Reverse transcription kit (Applied Biosystems, Carlsbad, CA, USA) and using primers and probe purchased from Applied Biosystems (Figure 1). Total RNA (15 ng/15 μl reaction) was converted to complementary cDNA for RNU6, mir-142-3p, let 7i, mir-125a-5p, mir-199b-5p, mir-193b, mir-365 and mir-125a-3p (Table 2) at the following protocols: 16℃ for 30 min, 42℃ for 30 min, 85℃ 5 min.

ABI primer probe system was used for identify specific miRNA. Stem-loop RT primer was used (Taqman miRNA assay, Applied Biosystems, Carlsbad, CA, USA) and this corresponds with specific miRNA.

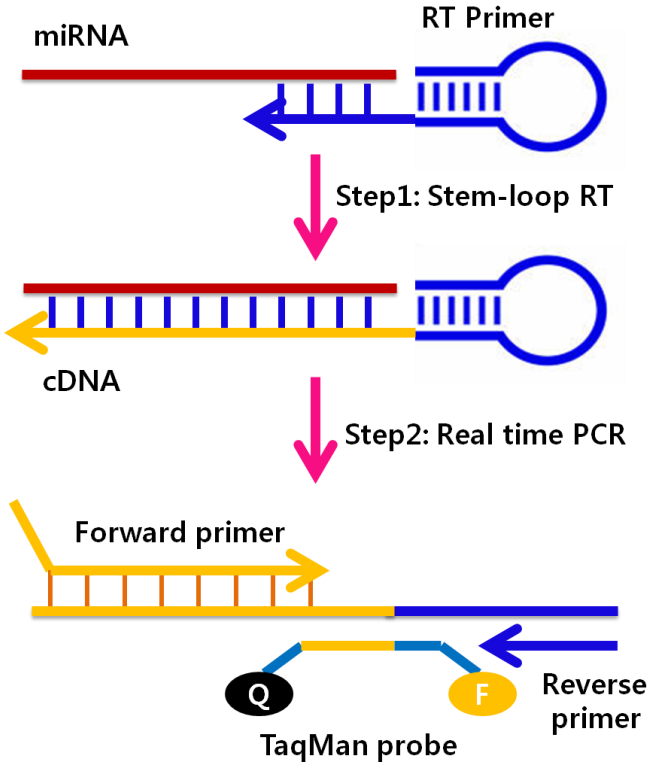

Figure 1. miRNA to reverse transcription synthesis

Table 2. Primer sequences for miRNA reverse transcription synthesis and qRT-PCR

assay name	Mature miRNA sequence
hsa-miR-125a-5p	UCCCUGAGACCCUUUAACCUGUGA
hsa-miR-365	UAAUGCCCCUAAAAAUCCUUAU
hsa-miR-199b-5p	CCCAGUGUUUAGACUAUCUGUUC
hsa-miR-193a-5p	UGGGUCUUUGCGGGCGAGAUGA
hsa-miR-125a-3p	ACAGGUGAGGUUCUUGGGAGCC
let-7i	UGAGGUAGUAGUUUGUGCUGU

4. Quantitative PCR and primers

A. mRNA quantitative PCR

Analyses on the mRNA expression were performed on 15 sporadic parathyroid tumors, 10 hereditary parathyroid tumors, 20 normal parathyroid gland tissues. Targets of specific miRNA in whole parathyroid samples, such as *CDC73* (*HRPT2*, parafibromin), *HES1*, *CCND1*, and *LIN7C* were analyzed by quantitative PCR along with housekeeping gene (beta actin). Roche Universal Probe Library Assay Design Center used for design to specific primer and probe in order to confirm the gene expressions. Quantitative PCR was performed with cDNA (5 ng) and primer (10 pmole) in an ABI 7500 quantitative PCR machine (Applied Biosystems, Carlsbad, CA, USA).

B. miRNA quantitative PCR

For miRNA expression quantification, 1 ng/ul cDNA of each sample were amplified with specific primers and probes for RNU6 and the mir-142-3p, let 7i, mir-125a-5p, mir-199b-5p, mir-193b, mir-365 and mir-125a-5p. miRNAs expression relative to RNU6 was calculated using the LivacMethod. Quantitative PCR was performed in ABI 7500 quantitative PCR machine (Applied Biosystems, Carlsbad, CA, USA).

5. Bioinformatics methods and statistical analysis

The Ingenuity Pathway Analysis (IPA) software (Ingenuity® Systems

version 8.0, www.ingenuity.com) was used to identify possible pathways associated to miR-199b-5p and *MENIN* gene. Statistical analyses were performed using SPSS 18.0 software (SPSS, Inc. Chicago, IL, USA). Differences in continuous variables between three groups were tested by one-way ANOVA with Tukey's post hoc-test, and between two groups were tested by independent-sample *t*-tests or Mann Whitney *U*-test. The *Spearman's* rank correlation coefficients were used to assess the associations between clinical and laboratory variables. A value of $P < 0.05$ was considered statistically significant.

III. RESULTS

1. Differences of clinical manifestations between hereditary and sporadic parathyroid tumors

We compared clinical and biochemical parameters of sporadic and hereditary parathyroid tumors. In patients with hereditary parathyroid tumor, tumor sizes were smaller and PTH and calcium levels were less high than those in the patients with sporadic forms (Table 3). We also confirmed germline mutations of the hereditary parathyroid tumors (Table 1).

Table 3. Clinical and biochemical characteristics

	Normal	Hereditary	Sporadic
Patient No.	20	10	15
Age	51.3 ± 10.1	48.2 ± 15.4	52.4 ± 11.9
Sex (M:F)	6:14	3:7	6:9
PTH (pg/mL)	31.7 ± 11.3	111.1 ± 43.4	233.0 ± 203.6*†
Ca (mg/dL)	9.3 ± 0.5	10.6 ± 0.9	11.4 ± 1.1*†
P (mg/dL)	3.7 ± 0.6	2.6 ± 0.4	2.6 ± 0.3*
Cr (mg/dL)	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.3
Tumor size (cm)		1.5 ± 0.8	2.0 ± 1.1

Data are mean ± SD.

One-way between-groups ANOVA with Tukey's post hoc-test.

*$P < 0.05$ *vs.* normal; †$P < 0.05$ sporadic *vs.* hereditary tumors.

2. miRNA cluster analysis

We analyzed miRNA expression in 3 sporadic and 3 hereditary parathyroid tumors normalized to 2 reference normal parathyroid tissues.

Supervised cluster analysis for differentially expressed 10 miRNA in hereditary and sporadic parathyroid tumor versus normal parathyroid tissues was shown (FDR < 0.05) (Figure 2). MiR-142-3p, let-7i, miR-125a-5p, miR-199b-5p, and miR-1274b_v16.0 were significantly up regulated, and miR-193b was down regulated in hereditay parathyroid tumor. Meanwhile, four miRNAs including miR-365, miR-125a-3p, miR-574-5p, and miR-1246 were significantly down regulated in sporadic parathyroid tumor. The mean fold-change (MF) of these miRNAs was calculated in Table 4.

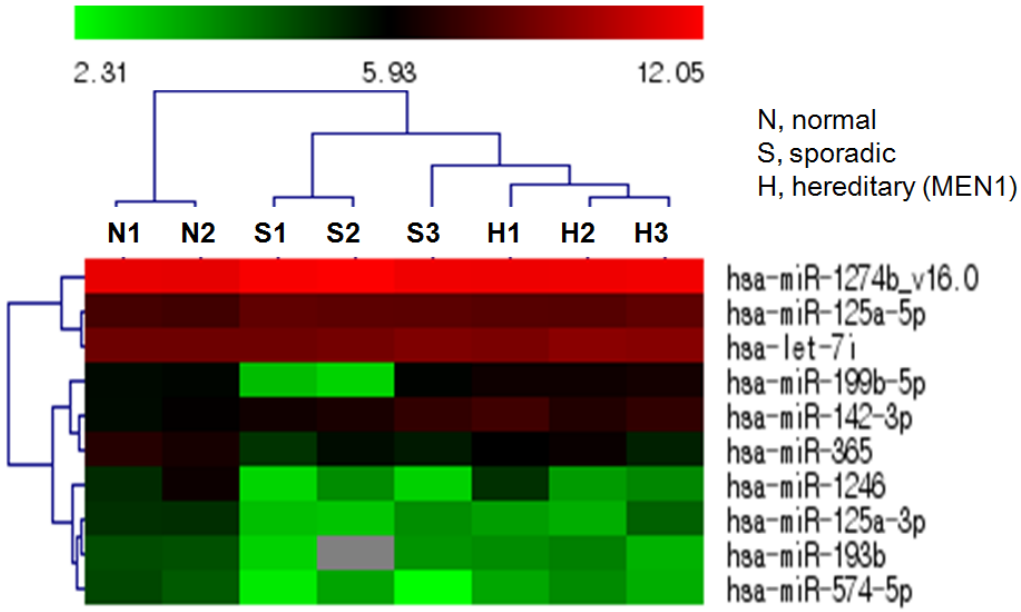

Figure 2. Supervised cluster analysis of miRNA levels in normal parathyroid tissues and parathyroid tumors. Heatmap of differentially expressed miRNAs. Data normalized to RNU6 were hierarchically clustered. Red indicates an *increase* relative to all data in this set; green indicates *a decrease* relative to all data in this set.

Table 4. miRNA fold changes in sporadic and hereditary parathyroid tumors detected by miRNA microarray.

miRNA	Hereditary parathyroid tumors /Normal parathyroid tissues expression ratio[a]	*P* value
miR-142-3p	**2.35**	**0.036**
let-7i	**1.46**	**0.044**
miR-125a-5p	**1.45**	**0.036**
miR-199b-5p	**1.44**	**0.010**
miR-1274b_v16.0	1.15	0.032
miR-193b	**0.51**	**0.043**
	Sporadic parathyroid tumors /Normal parathyroid tissues expression ratio[a]	
miR-365	**0.45**	**0.042**
miR-125a-3p	**0.29**	**0.017**
miR-574-5p	0.28	0.042
miR-1246	0.17	0.047

[a]Ratio of median expression levels sorted in descending order

miRNAs in bold were validated in 20 normal parathyroid tissues, 10 hereditary and 15 sporadic parathyroid tumors.

3. Validation of miRNA expression by RT-PCR analysis

We identified 10 differentially expressed miRNAs between hereditary and sporadic versus normal parathyroid tissues. Seven commercially available miRNAs were used to validate the miRNA array expression data by real-time quantitative PCR (Figure 3). As a result, miR-193b and miR-365 were significantly down-regulated and miR-125a-3p and let-7i were up-regulated in sporadic parathyroid tumors. MiR-125a-3p expression evaluated by qRT-PCR was different from the microarray result. Of note, only miR-199b-5p showed significantly different expression between two parathyroid tumor types that it

was up-regulated in hereditary form but down-regulated in sporadic form.

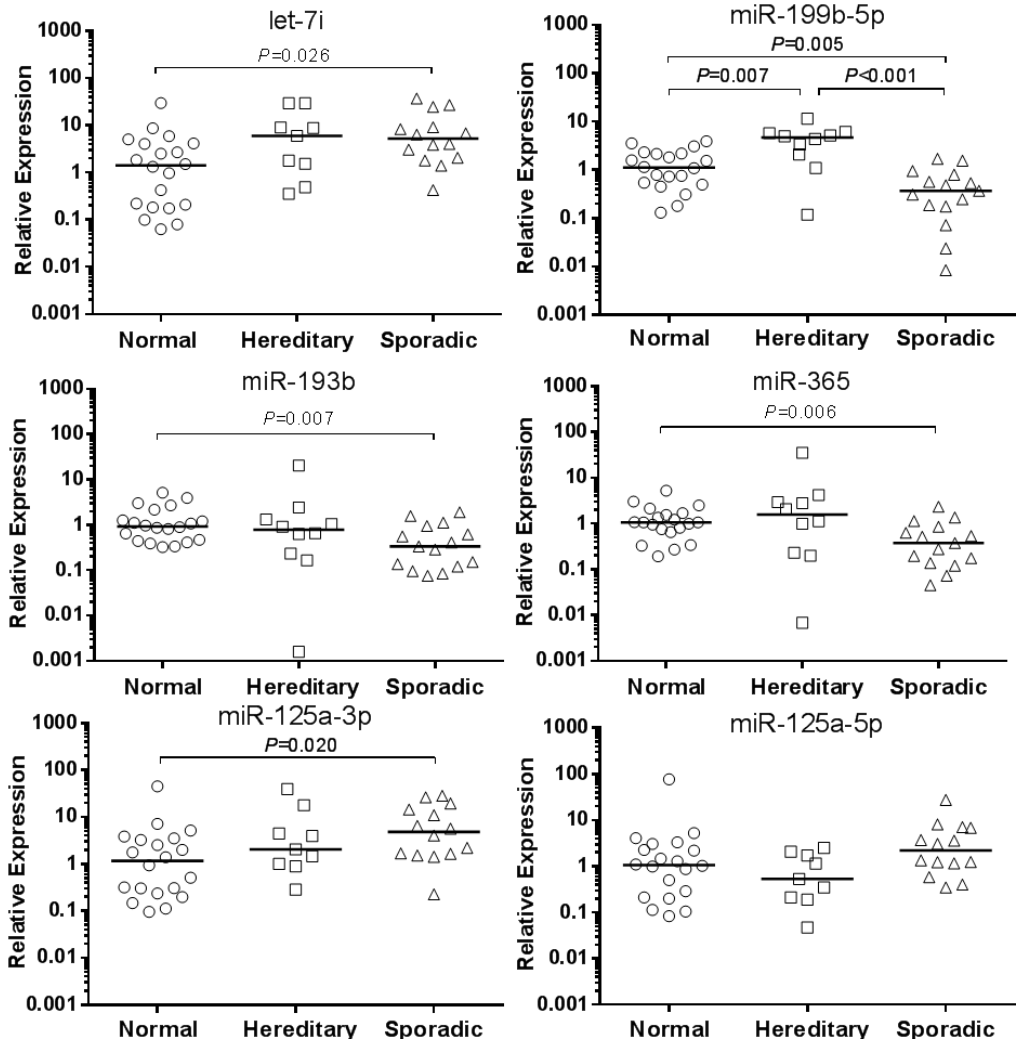

Figure 3. Differential expressions of specific miRNAs in parathyroid tumors. Compared with normal parathyroid tissues (n = 20), levels of miRNAs expression in hereditary (n = 10) and sporadic (n = 15) parathyroid tumors were determined by Taqman quantitative RT-PCR analysis. Horizontal bars represent the median values.

4. Correlation between aberrant expression of miR-199b-5p and PTH level

PTH level was correlated with tumor size, significantly in sporadic parathyroid tumors (γ = 0.536, P = 0.040), but not significantly in MEN 1-related form (γ = 0.280, P = 0.432). Of interest, different correlations between PTH level and miR-199b-5p according to types of parathyroid tumor were identified, negative correlation in sporadic parathyroid tumors (γ = –0.718, P = 0.003), but positive association in hereditary form (γ = 0.430, P = 0.214) (Figure 4).

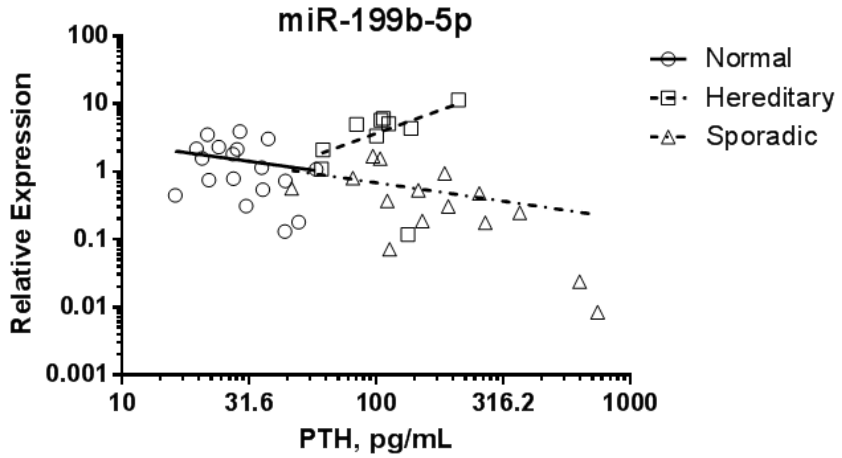

Figure 4. Different correlation between serum PTH level and relative expression of miR-199b-5p in parathyroid tumors. A negative correlation was found between miR-199b-5p and PTH level in normal parathyroid tissues (γ=-0.356, P=0.147) and sporadic parathyroid tumors (γ=-0.718, P=0.003), but positive correlation in MEN 1-related parathyroid tumors (γ=0.430, P=0.214).

As expected, *MENIN* mRNA expression was significantly down-regulated in sporadic and hereditary parathyroid tumor versus normal parathyroid tissue (P = 0.008 in sporadic form versus P = 0.001 in hereditary form) (Figure 5A). *MENIN* mRNA showed negatively correlation with PTH level in normal parathyroid tissues and parathyroid tumors with sporadic and MEN 1-related forms ($\gamma = -0.408$, P = 0.007) (Figure 5B).

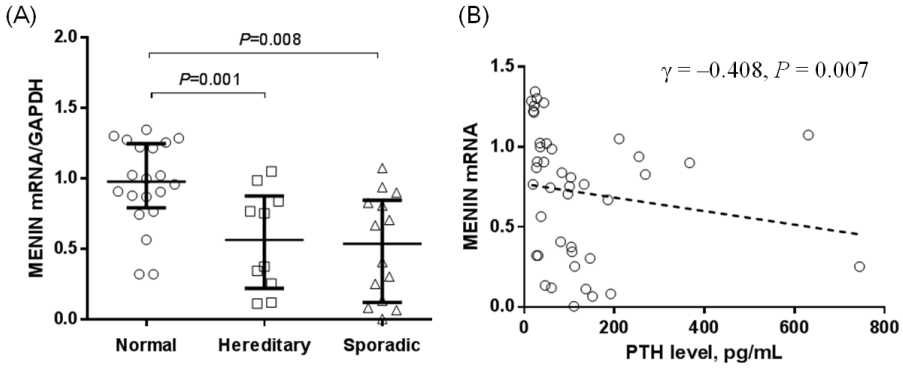

Figure 5. Representative mRNA expression of the *MENIN* gene in parathyroid tumors (A) and correlation with PTH. Horizontal bars represent the median values with inter-quartile range.

5. Altered parathyroid tumor related gene expression by hereditary and sporadic forms

The relative mRNA expression levels of *LIN7C, CCND1, CDC73,* and *HES1* which were associated with parathyroid tumors [1,12] or also known as a target of miR-199b-5p [13] were determined by real-time quantitative RT-PCR in 15 sporadic and 10 MEN 1-related parathyroid tumors (Figure 6). We

found that *CCND1* and *CDC73* mRNA were significantly increased only in MEN 1-related parathyroid tumors, but could not find correlations with miR-199b-5p.

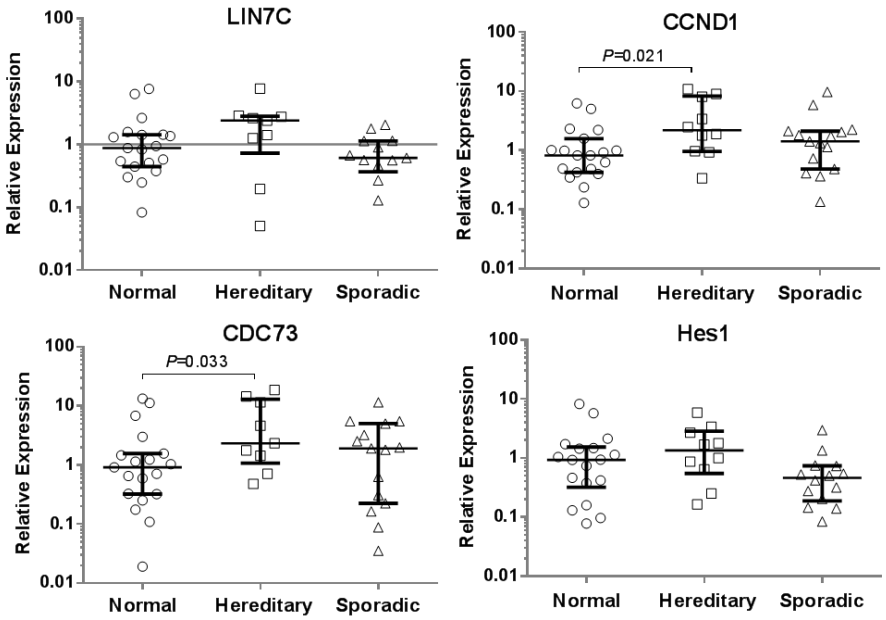

Figure 6. *LIN7C*, *CCND1*, *CDC73*, and *HES1* mRNA expression in hereditary and sporadic parathyroid tumors. Horizontal bars represent the median values with inter-quartile range.

6. Network between miR-199b-5p and *MENIN*

Although miR-199b-5p was differentially expressed between sporadic and MEN 1-related parathyroid tumors, we could not find direct relationship between miR-199b-5p and *MENIN* mRNA in the experimental setting. Therefore, we predicted a network between miR-199b-5p and *MENIN* gene

bioinformatically, using IPA gene network software. Of interest, one pathway was identified with known relevance for “Gene expression, cellular development, cellular growth and proliferation” (Figure 7). The top disease and bio-function of this analysis are “Cancer” (p value = 1.15E-04 ~ 4.61E-02) and “Cell Cycle” (p value= 1.15E-04 ~ 4.19E-02), respectively.

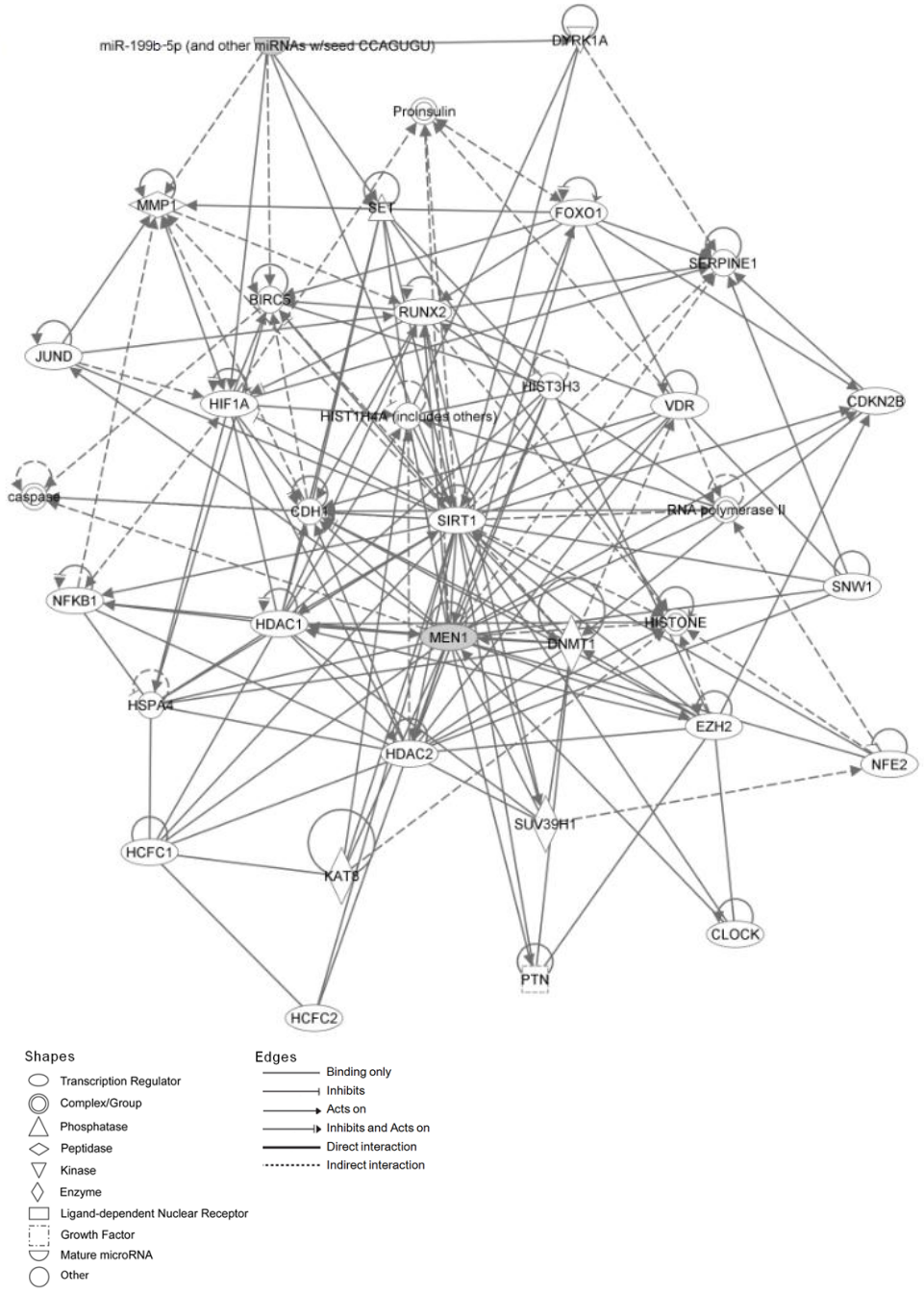

Figure 7. A network predicted to be regulated by miRNA-199b-5p and *MENIN* gene. One predicted network regulated by miRNA-199b-5p and *MENIN* gene was "Gene expression, cellular development, cellular growth and proliferation".

IV. DISCUSSION

Little is known about exact molecular mechanism between sporadic and hereditary parathyroid tumors, explaining the different profiles of disease progression and phenotypes. In the present work, we found miR-199b-5p was differentially expressed between sporadic and hereditary parathyroid tumors and correlated otherwise with PTH.

Several studies reported that the volume or weight of parathyroid adenoma, most common form of sporadic parathyroid tumors, was significantly correlated with PTH levels [14,15], as also observed in the present study. Parathyroid adenoma is part of a spectrum of parathyroid proliferative disorder, which is consisted of a monoclonal proliferation of a single cell type, mainly chief cell which synthesize and secrete PTH [16]. Therefore, PTH level could reflect the degree of cell proliferation in parathyroid tumors. Meanwhile, MEN 1-related parathyroid tumors are characterized by wide range of variability in size and cytoarchitectures from normal to diffuse and/or nodular hyperplasia and, occasionally, to adenoma-like tumors [16]. This wide range of histological abnormalities are the result of polyclonal cell expansion of MEN 1 parathyroid gland [17,18], and could explain less correlation between tumor size and PTH in MEN 1-related parathyroid tumors. However, there is no specific morphopathological difference between polyclonal and monoclonal parathyroid tumors either

caused by hereditary or sporadic parathyroid tumors [19].

Several responsible germline genetic changes associated with parathyroid tumors in familial syndrome have been identified such as *MENIN* in MEN 1, *RET* in MEN 2A, and *CDC73/HRPT2* in HPT-JS (hyperparathyroidism jaw tumor syndrome) [20,21]. However, these genetic alterations also have been implicated only in a subset of sporadic parathyroid tumors. Genetic alterations in the *MENIN* gene have been also reported in 20 to 30% of sporadic parathyroid tumors [22]. Therefore, the presence constitutionally mutated *MENIN* alleles does not seem to be sufficient for development of parathyroid tumors. The *MENIN* gene would function as a tumor suppressor gene that underlies tumorigenesis in MEN 1 based on the loss of function of the wild-type allele by a somatic alteration such as loss of heterozygosity (LOH) or inactivating mutation, as predicted by the two hit model of Knudson [18,23,24]. Interestingly, emerging evidence shows that even partial inactivation of tumor suppressors can critically contribute to tumorigenesis, proposed as a continuum model of tumor suppressor gene function [8]. In this context, miRNAs can be a good candidate as controlling subtle regulation of gene expression and/or activity and its involvement in the tumorigenesis. Interestingly, this presumption was proved, in part, by Luzi *et al.* showing that miR-24-1 targeting *MENIN* mRNA mimics the second somatic “hit” of *MENIN* gene inactivation and its potential function to fine-tune gene expression with a negative feedback loop between miR-24-1 and

Menin [25].

Substantial advances in the study of the involvement of miRNAs on parathyroid tumorigenesis have been achieved in recent years. Since Costa-Guda *et al.* have reported preliminary data that miR-15a and miR-16-1 genes are frequently deleted in human parathyroid carcinoma [26], Corbetta *et al.* analyzed the differential expression of miR-296, miR-139, miR-503, and miR-222 in human parathyroid carcinomas in comparison with normal parathyroid tissue [27]. Recently, Rahbari *et al.* showed that miR-26b, miR-30b, and miR-126 were significantly dysregulated between parathyroid carcinoma and adenoma [11]. There studies suggest the existence of an altered miRNA expression pattern in parathyroid carcinomas compared to normal or parathyroid adenoma.

In the present work, we found miR-199b-5p was differentially expressed between sporadic and hereditary parathyroid tumors. Among an expanding studies related miRNAs, miR-199b-5p is a putative tumor suppressor targeting several signaling pathways: *HES1* involved in SHH and Notch pathway in medulloblastoma [28] and osteosarcoma [29], *PODXL* and *DDR1* in acute myeloid leukemia [30], the nuclear kinase Dyrk1a in heart [31], *HIF1a* in hepatocellular carcinoma [32], and *HER2* and its downstream signaling ERK1/2 and AKT pathway in breast cancer [33]. Taken together, overexpression of miR-199b-5p could significantly inhibit cell proliferation, migration, and clonogenicity. A quite interesting point on the miR-199b-5p in

medulloblastoma is that its expression changes as the diseases transforms into more malignant stages [32]. The study suggested miR-199b-5p can be a fine tuner of the levels of their target *HES1* involved in notch pathway and suggested its epigenetic control function during the tumor development [13].

Regarding the anti-proliferative function of miR-199b-5p, it is conceivable that differentially expressed miR-199b-5p between sporadic and hereditary parathyroid tumors in the present study could explain the different clinical features between two types of parathyroid tumors. Under the condition of low Menin as described in this study, in sporadic and hereditary parathyroid tumors, up-regulation of miR-199b-5p in MEN 1-related parathyroid tumors, in part, could repress tumor proliferation induced by several proliferative genes due to de-suppressive effect of low Menin. Our data regarding clinical and biochemical characteristics of cases with MEN 1-related parathyroid tumors, smaller tumor size and lower levels of PTH and Ca than sporadic forms, might support this hypothesis.

The network analysis demonstrated the potential for miR-199b-5p and *MENIN* gene to operative together directly/indirectly in the biological pathway with relevance for cancer. According to IPA, miR-199b-5p directly target transcription regulators, *HIF1A* and *SIRT1*, which are also known interactants with *MENIN* gene [34,35]. Indeed, PTH have been reported as being regulated by Menin [36], and our data showed significant correlation between PTH and miR-199b-5p level in parathyroid tumors. However, these

complex relations have to be proved in further investigations.

In the light of our data on the correlation between PTH and miR-199b-5p which differentially expressed in sporadic and hereditary parathyroid tumors, it will be of interest to focus future studies on the role of miR-199b-5p in parathyroid tumorigenesis. Further functional studies are warranted.

V. CONCLUSION

In summary, we identified that miR-199b-5p was differentially expressed between sporadic and MEN 1-related parathyroid tumors and correlated otherwise with PTH. Using integrative bioinformatics, the network between miR-199b-5p and *MENIN* gene is speculated. Down-or up-regulation of miRNA-199b-5p seems to have a distinct role in the tumorigenesis leading to the phenotypic differences in sporadic and hereditary types of parathyroid tumors. This argues for the involvement of miRNAs with different manner in the pathogenesis of parathyroid tumors under different genetic background.

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ABSTRACT (IN KOREAN)

산발성 부갑상선 종양 및 유전성 부갑상선 종양에서 다르게 발현되는 microRNA의 규명

<지도교수 이 유 미>

연세대학교 대학원 의과학과
정 윤 정

배경:

miRNA는 종양발생에서 유전자 발현을 조절하는 역할을 하는 것으로 알려져 있다. 그러나 다른 유전학적 배경을 가진 각각의 부갑상선종양의 miRNA 표현양상에 대한 연구는 거의 없다. 본 연구에서는 종양발생의 분자유전학적 특성을 잘 이해하기 위해 유전성 부갑상선 종양과 산발성 부갑상선 종양에서의 miRNA 발현 양상과 임상적 특성의 상관관계를 보고자 하였다.

방법:

6명의 부갑상선 종양 (3명은 산발성 부갑상선 종양, 3명은 MEN 1 환자의 부갑상선 종양)과 2개의 정상 부갑상선조직의 RNA를 추출하여 887개의 인간 miRNA를 포함하는 miRNA array를 수행하였다. 발현차이를 보이는 miRNA는 RNU6 로 보g정하여 15개의 산발성 부갑상선 종양과 10개의 MEN 1 부갑상선 종양과 20개의 정상 부갑상선 조직과의 상대적 표현을 정량하였다.

결과:

Microarray 를 통해서 부갑상선 정상조직과 산발성 또는 MEN 1 부갑상선 종양 간 의미 있게 (FDR < 0.05) 발현차이를 보이는 10개의 miRNA를 확인하였다. 이 중 7개의 miRNA를 검증하였는데 종양억제유전자 작용으로 알려져있는 miR-199b-5p 만이 산발성 부갑상선 종양과 MEN 1 부갑상선 종양 간 의미있게 발현차이를 보였다. 또한 miRNA 199b-5p는 산발성 부갑상선 종양에서는 PTH 수치와 의미있게 음의 상관관계를 보였고 MEN 1 부갑상선 종양에서는 유의하지는 않았지만 양의 상관관계를 보였다. 종양의 특징을 비교해 볼 때 MEN 1 부갑상선 종양에 비해 산발성 부갑상선 종양의 크기가 더 크고 PTH 가 더 증가되어 있어, miR-199b-5p의 발현차이와 어느 정도 연관성이 있을 수 있다. MEN1 유전자와 miR199b-5p와는 시그날 예측모델에서 세포성장 및 세포증식과 관련되어 종양발생에 연결성을 생각해 볼 수 있었다.

결론:

miRNA-199b-5p 의 경우, 산발성 부갑상선 종양에서는 감소되고, 유전성 부갑상선 종양에서는 증가되어 있는 발현차이를 보여 이 두 종양의 서로 다른 표현형질과 연관되어 있을 가능성을 보여주었다. 향후 어떤 기전으로 조절이 되는지에 대한 분자생물학적 연구가 필요하겠다.

핵심되는 말 : miRNAs, 다발성 내분비 종양 1 형, 부갑상선 종양