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The Experimental Study of miRNA in Pituitary Adenomas

Pitüiter Adenomlarda miRNA Deneysel Çalışması

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ABSTRACT

AIM: We investigated the differential miRNA expression in pituitary adenomas (both non-functioning and gonadotropin-secreting) and normal pituitaries.

MATERIAL and METHODS: RNA was extracted and purified from pituitary adenomas (10 non-functioning and 10 gonadotropin-secreting) and from two normal pituitary tissue samples. The samples were analyzed by miRNA microarray. Gene expression was measured using real-time RT-PCR with SYBR GREEN I.

RESULTS: In non-functioning pituitary adenomas, 25 miRNA genes were up-regulated (six by over 5-fold) and 15 were down-regulated (six by more than 10-fold). miR-124a was the most up-regulated gene (38.58-fold), and miR-31 the most down-regulated gene (21.5-fold). In gonadotropin-secreting pituitary adenomas, 16 miRNA genes were up-regulated (six by over 4-fold) and 13 were down-regulated (seven by more than 10-fold). miR-10b was the most up-regulated gene (48.73-fold), and miR-503 the most down-regulated gene (39.8-fold). Five genes were up-regulated in both subtypes: miR-523, miR-10b, miR-520b, miR-422a, and miR-422b. The RT-PCR results were consistent with those of the gene chips.

CONCLUSION: We established miRNA expression maps of non-functioning and gonadotropin-secreting pituitary adenomas. The most strongly differentially expressed genes were miR-124a and miR-31 in non-functioning pituitary adenomas, and miR-10b and miR-503 in gonadotropin-secreting pituitary adenomas.

KEYWORDS: Pituitary adenoma, miRNA gene, Gene chip

ÖZ

AMAÇ: Pitüiter adenomlarda (hem çalışmayan hem gonadotropin salan) ve normal hipofiz bezlerinde diferansiyel miRNA ekspresyonunu araştırdık.

YÖNTEM ve GEREÇLER: RNA pitüiter adenomlardan (10 çalışmayan ve 10 gonadotropin salan) ve iki normal pitüiter doku örneğinden ekstrakte edilip saflaştırıldı. Örnekler miRNA mikroarray ile analiz edildi. Gen ekspresyonu SYBR GREEN I ile gerçek zamanlı RT-PCR kullanılarak ölçüldü.

BULGULAR: Çalışmayan pitüiter adenomlarda 25 miRNA geni yukarı doğru düzenlenmişti (altısı 5 kattan fazla) ve 15'i aşağı düzenlenmişti (altısı 10 kattan fazla). miR-124a en çok yukarı düzenlenmiş gendi (38,58-kat) ve miR-31 en çok aşağı düzenlenmiş gendi (21,5-kat). Gonadotropin salan pitüiter adenomlarda 16 miRNA geni yukarı doğru düzenlenmişti (altısı 4 kattan fazla) ve 13'ü aşağı düzenlenmişti (yedisi 10 kattan fazla). miR-10b en çok yukarı düzenlenmiş gendi (48,73-kat) ve miR-503 en çok aşağı düzenlenmiş gendi (39,8-kat). Beş gen her iki alt tipte yukarı düzenlenmişti: miR-523, miR-10b, miR-520b, miR-422a ve miR-422b. RT-PCR sonuçları gen çiplerininkiyle tutarlıydı.

SONUÇ: Çalışmayan ve gonadotropin salan pitüiter adenomların miRNA ekspresyon haritalarını oluşturduk. En kuvvetli ayırıcı bir şekilde ekspresyon görülen genler, çalışmayan pitüiter adenomlarda miR-124a ve miR-31 ve gonadotropin salan pitüiter adenomlarda miR-10b ve miR-503 oldu.

ANAHTAR SÖZCÜKLER: Pitüiter adenom, miRNA geni, Gen çipi

INTRODUCTION

Pituitary adenomas occur with a high incidence and account for about 10-15% of all cranial tumors. The growth pattern of these tumors makes them very difficult to completely remove, and some patients with pituitary adenomas may suffer recurrence. Improved treatments depend on further exploration of pituitary adenomas pathogenesis. The protooncogenes and anti-oncogenes of pituitary adenomas have been studied for several years (6,16,20), but the pathogenesis remains unclear.

The discovery of microRNAs has provided a new way of studying pituitary adenomas. MicroRNAs (miRNAs) are an extensive class of short non-coding RNAs that can play important regulatory roles in an organism by targeting mRNAs for cleavage or translational repression. Mature miRNAs are 19- to 25-nucleotide-long molecules cleaved

from 70- to 100-nucleotide hairpin pre-miRNA precursors. The precursors are cleaved by cytoplasmic RNase III Dicer into 22-nucleotide miRNAs. The discovery of miRNA reveals a new level of gene regulation for processes such as differentiation, cell growth, and cell death (26). Deviations from normal expression patterns may play a role in diseases. It has been shown that miRNAs are aberrantly expressed or mutated in human cancers, suggesting that they may represent a novel class of oncogenes or tumor suppressor genes (8,17,22,32). However, as a kind of important tumorigenesis (23), to date, there has been very little research about the association between miRNA and pituitary adenomas.

Here we investigated the relationship between miRNA and pituitary adenomas. We performed genome-wide miRNA expression profiling in pituitary adenomas and normal pituitaries. Our results demonstrate the existence of a pituitary adenoma-specific miRNA signature.

MATERIAL and METHODS

Pituitary Adenomas and Normal Pituitary Samples

Pituitary adenomas samples (10 non-functioning pituitary adenomas and 10 gonadotropin-secreting pituitary adenomas) were obtained from the Department of Neurosurgery of Tianjin Huanhu Hospital, where they were freshly resected during surgery and immediately frozen in liquid nitrogen for subsequent total RNA extraction. Two normal pituitary samples were obtained from Tianjin Medical University. RNA was isolated from tissues using Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Microarray Experiment

From each sample, miRNAs were separated from 50-100 µg total RNA with Ambion's miRNA Isolation Kit. Fluorescence labeling with T4 RNA ligase was performed using 25 µg miRNA PEG, 0.1 mM ATP, 50 mM HEPES, 3.5 mM DDT, 20 mM $MgCl_2$, 10 mg/ml BSA, 10% DMSO, 500 mg 5'-phosphate-cytidyl-uridyl-Cy3-3' (Dharmacon), and 20 µg T4 RNA ligase.

Each miRNA profiling microarray was hybridized with 1.5 µg biotinylated miRNA in 16 µl formamide prehybridization/hybridization solution (0.2% SDS; 3× SSC; 50× Denhardt's solution) at 42°C overnight. The hybridized microarray was washed with 2× SSC/0.2% SDS at 42°C for 4 min, then washed with 0.2% SDS at room temperature for 4 min. After thorough washing, the microarray was scanned on a LuxScan 10K/A Scanner.

SmartArray™ microarrayer (CapitalBio Corp., Beijing, China) contained 469 probes in triplicate, including 435 human miRNA genes and 261 mouse miRNA genes (with some sequences overlapping between human and mouse). U6 was used as an internal control. Zip5, Zip13, Zip15, Zip21, Zip23, Zip25,Y2, and Y3 were used as external controls. Hex was used as positive contrast, and 50% DMSO was used as negative contrast.

Data Analysis

Images of the miRNA microarray were transformed into digital signals using GenePix Pro 4.0 (Axon Instruments); all bad dots and weak dots were deleted. Differentially expressed genes were identified via Significance Analysis of Microarrays (SAM, Version 2.1); FDR was controlled to be less 5%, and a 1.5-fold change was considered significant.

Quantitative RT-PCR for miRNA

Quantitative RT-PCR was performed to verify the results for the miRNA chips of miR-124a and miR-10b, which were the most up-regulated in the gene expression maps of the non-functioning pituitary adenomas and gonadotropin-secreting pituitary adenomas. Briefly, RNA was reverse transcribed to cDNA with gene-specific primers, and the amount of each miRNA relative to U6 RNA for initiator methionine was described using the following equation:

$$ratio = \frac{(E_{target})^{\Delta CP_{target}(control-sample)}}{(E_{ref})^{\Delta CP_{ref}(control-sample)}}$$

RESULTS

To investigate whether miRNAs are differently expressed in pituitary adenomas versus normal pituitary tissue, we performed microarray analysis to examine global expression levels of 469 miRNAs.

In the non-functioning pituitary adenomas, 25 miRNA genes were up-regulated, and 15 were down-regulated (Table I). Six genes were up-regulated over 5-fold: miR-124a, miR-146a, miR-523, miR-10b, PREDICTED_MIR240, PREDICTED_MIR207 (the last two were predicted by the computer program). Six miRNA genes were down-regulated more than 10-fold: miR-31, miR-514, miR-503, miR-506, miR-513, and miR-218. miR-124a was the most up-regulated gene, while miR-31 was the most down-regulated gene.

In the gonadotropin-secreting pituitary adenomas, 16 miRNA genes were up-regulated, and 13 were down-regulated (Table II). Six genes were up-regulated over 4-fold: miR-10b, miR-523, miR-222, miR-422b, PREDICTED_MIR189, and PREDICTED_MIR240 (the last two were predicted by the computer program). Seven miRNA genes were down-regulated more than 10-fold: miR-503, miR-508, miR-514, miR-31, miR-506, miR-513, and miR-509. miR-10b was the most up-regulated gene, while miR-503 was the most down-regulated gene.

DISCUSSION

miRNAs constitute a recently discovered class of small non-coding RNAs that regulate target gene expression either by decreasing the stability of the target mRNA or by translational inhibition. They are involved in diverse processes, including cellular differentiation, proliferation, and apoptosis. Recent evidence also suggests their importance in carcinogenesis.

Bottoni et al. (3) analyzed miR-15a and miR-16-1 expression in 10 GH-secreting and 10 PRL-secreting pituitary

Table I: miRNA Gene of Nonfunctional Pituitary Adenoma

Row	Gene ID	Gene Name	Score(d)	Fold change
Positive Genes				
18	hsa-miR-124a	hsa-miR-124a	1.614718895	38.58056441
40	hsa-miR-146a	hsa-miR-146a	3.060045284	6.806237515
202	PREDICTEDMIR2	PREDICTEDMIR240	4.95505464	6.466669895
181	hsa-miR-523	hsa-miR-523	5.275022728	5.972007501
17	hsa-miR-10b	hsa-miR-10b	1.747788897	5.746391476
200	PREDICTEDMIR207	PREDICTEDMIR207	4.255095108	5.107561483
60	hsa-miR-182	hsa-miR-182	4.266143932	4.918530063
201	PREDICTEDMIR220	PREDICTEDMIR220	1.559610113	4.779870474
177	hsa-miR-520b	hsa-miR-520b	3.987462518	4.285677898
191	PREDICTEDMIR112	PREDICTEDMIR112	5.878891143	4.245945076
38	hsa-miR-144	hsa-miR-144	3.491440219	3.967710079
139	hsa-miR-373	hsa-miR-373	2.875070502	3.962348212
152	hsa-miR-422b	hsa-miR-422b	2.650071041	3.834670124
79	hsa-miR-202	hsa-miR-202	3.760392566	3.606820086
179	hsa-miR-520e	hsa-miR-520e	3.507228182	3.573491606
115	hsa-miR-32	hsa-miR-32	5.108618714	3.550253572
151	hsa-miR-422a	hsa-miR-422a	3.528629286	3.508553774
57	hsa-miR-181c	hsa-miR-181c	1.707285804	3.36650496
199	PREDICTEDMIR206	PREDICTEDMIR206	3.508375674	3.315085604
56	hsa-miR-181b	hsa-miR-181b	4.244015013	3.215265938
178	hsa-miR-520c	hsa-miR-520c	1.561473293	3.111472203
194	PREDICTEDMIR166	PREDICTEDMIR166	3.932279496	3.071632012
64	hsa-miR-188	hsa-miR-188	3.237736956	3.065099271
49	hsa-miR-155	hsa-miR-155	4.364986788	3.064296396
180	hsa-miR-520f	hsa-miR-520f	1.750024494	3.029377021
Negative Genes				
114	hsa-miR-31	hsa-miR-31	-7.1914409	0.046479541
175	hsa-miR-514	hsa-miR-514	-5.4507773	0.051961002
169	hsa-miR-503	hsa-miR-503	-5.2348814	0.05450016
171	hsa-miR-506	hsa-miR-506	-5.9005418	0.058479025
174	hsa-miR-513	hsa-miR-513	-7.1425836	0.069682416
89	hsa-miR-218	hsa-miR-218	-5.8596041	0.071529287
173	hsa-miR-509	hsa-miR-509	-8.7041487	0.109461549
73	hsa-miR-199b	hsa-miR-199b	-7.4520557	0.112344374
172	hsa-miR-508	hsa-miR-508	-4.3094042	0.115720551
161	hsa-miR-489	hsa-miR-489	-11.533573	0.155048176
86	hsa-miR-212	hsa-miR-212	-7.640375	0.199926246
163	hsa-miR-493	hsa-miR-493	-3.4194304	0.236512421
158	hsa-miR-450	hsa-miR-450	-3.5828627	0.241049575
133	hsa-miR-363	hsa-miR-363	-3.1465249	0.277146075
154	hsa-miR-424	hsa-miR-424	-3.307936	0.315590774

Table II: miRNA Gene of Pituitary Gonadotroph Adenoma

Row	Gene ID	Gene Name	Score(d)	Fold change
Positive Genes				
17	hsa-miR-10b	hsa-miR-10b	6.080628868	48.7256298
192	PREDICTEDMIR189	PREDICTEDMIR189	3.135308919	21.76285068
172	hsa-miR-523	hsa-miR-523	3.377142912	6.154020582
87	hsa-miR-222	hsa-miR-222	2.097596422	4.526587822
144	hsa-miR-422b	hsa-miR-422b	8.598189023	4.415722457
197	PREDICTEDMIR240	PREDICTEDMIR240	3.134414598	4.379202854
196	PREDICTEDMIR207	PREDICTEDMIR207	3.401952897	3.989775885
167	hsa-miR-520b	hsa-miR-520b	2.2225657	3.769846744
143	hsa-miR-422a	hsa-miR-422a	8.650877463	3.766147319
32	hsa-miR-139	hsa-miR-139	4.367513674	3.550021035
121	hsa-miR-34a	hsa-miR-34a	8.669206454	3.468870276
195	PREDICTEDMIR206	PREDICTEDMIR206	2.098021484	3.325045756
86	hsa-miR-221	hsa-miR-221	2.178178703	3.306058787
189	PREDICTEDMIR166	PREDICTEDMIR166	2.181959724	3.101718557
186	PREDICTEDMIR112	PREDICTEDMIR112	2.674281152	3.073572431
182	mmu-miR-140	mmu-miR-140	8.154494137	3.049278124
Negative Genes				
160	hsa-miR-503	hsa-miR-503	-7.406263999	0.025126059
162	hsa-miR-508	hsa-miR-508	-9.579973277	0.031155365
165	hsa-miR-514	hsa-miR-514	-6.067098906	0.04078874
110	hsa-miR-31	hsa-miR-31	-8.988899776	0.044040174
161	hsa-miR-506	hsa-miR-506	-7.800728636	0.051745326
164	hsa-miR-513	hsa-miR-513	-6.35651444	0.056497864
163	hsa-miR-509	hsa-miR-509	-5.951729669	0.059794064
149	hsa-miR-450	hsa-miR-450	-8.555721112	0.101689101
69	hsa-miR-199b	hsa-miR-199b	-6.863310473	0.102111601
125	hsa-miR-363	hsa-miR-363	-10.61248254	0.152319382
155	hsa-miR-493	hsa-miR-493	-3.448521884	0.156901676
153	hsa-miR-489	hsa-miR-489	-6.231848181	0.228087592
146	hsa-miR-424	hsa-miR-424	-7.505464268	0.299777161

macroadenomas by northern blot; they found that both miRNAs are expressed at lower levels in pituitary adenomas compared to normal pituitary tissue, and that their expressions inversely correlate with tumor diameter. Two years later, his further study suggested that miRNAs could be useful to identify the classification of pituitary adenoma histotypes and differentiate micro- from macro-adenomas (4).

Mao et al. (19)compared GH-secreting pituitary adenomas with normal pituitaries by miRNA microarray; they found that 52 miRNAs were differentially expressed (23 up-regulated and 29 down-regulated), and miR-136 and miR-125b were the most strongly differentially expressed genes. Stilling et al. (28) used a miRNA microarray with 1,145 probes to study miRNA expression in six normal pituitaries and eight ACTH pituitary adenomas; 188 genes were up-regulated, 160 were down-regulated, and the most strongly differentially expressed genes were miR-122 and miR-493. Amaral et al. (1) studied the differential expression of let-7a, miR-15a, miR-16, miR-21, miR-141, miR-143, miR-145, and miR-150 in ACTH-secreting pituitary tumors and normal pituitary to investigate the relationship between their expression profiles and tumor size or remission after treatment; they found no associations between miRNA expression and tumor size or ratio of remission after surgery. Shi et al. (25) has summarized the relationship between the miRNA and pituitary adenomas. For example, miR-21 can regulate the growth and invasion of pituitary tumors by regulating its target genes, as the tumor suppressor genes of pituitary adenoma. On the other

hand, miR-15a and miR-16 potentiate the normal apoptotic response by targeting the antiapoptotic gene BCL-2, and other miRNAs expression in the GH-secreting adenomas, PRL-secreting adenomas and adrenocorticotropic hormone (ACTH)-secreting pituitary adenomas. At last, he concluded that miRNAs can be used as a potential agent to treat pituitary adenomas. Overall, very little is yet known about the importance of miRNA in pituitary adenomas.

In the present study, we found that brain-specific miR-124a was most differentially expressed in non-functioning pituitary adenomas compared to normal pituitaries; it was strongly expressed in non-functioning pituitary adenomas but not expressed in normal pituitaries. miR-124a is reportedly very important for brain development. Krichevsky et al. (15) studied the miRNA expression profiles during normal brain development, and found that the miR-124a signal increased more than 13-fold from E12 to E21, after which it remained stable. Smirnova et al. (27) studied the regulation of expressed neural miRNA during brain development, and found miR-124a to be one of the most highly expressed miRNAs in the adult brain. As a specifically expressed miRNA in the human brain, miR-124a was found in cerebrum, cerebellum, and brain stem. Our results showed miR-124a expression exclusively in non-functioning pituitary adenomas, suggesting that miR-124a is an oncogene of non-functioning pituitary adenomas in addition to its role in neuron development. Thus, this miRNA may be a double-edged sword for the human brain. Butz et al. (5) also studied the miRNA expression profiles in non-functioning pituitary adenomas; their results differ from ours, further highlighting the complexity of miRNA actions within the nervous system.

We found that miR-10b was the most differentially expressed miRNA in gonadotropin-secreting pituitary adenomas; it was over-expressed in gonadotropin-secreting pituitary adenomas compared to both normal pituitaries and non-functioning pituitary adenomas. Davoren et al. (7) found miR-10b to be the most significantly deregulated miRNA in breast cancer, and Gabriely et al. (10) reported specific over-expression of miR-10b in glioblastoma cells. Bentwich et al. (2) showed that miR-10b was over-expressed in human testis and prostate, further showing the relationship of miR-10b with sex hormones.

We found that miR-222 was up-regulated 4.5-fold in gonadotropin-secreting pituitary adenomas compared to normal pituitaries. miR-222 is located on the X chromosome and is reportedly over-expressed in placenta, cerebrum, and prostate (2). Kasashima et al. (14) studied miRNAs during 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced differentiation of human leukemia cells (HL-60) into monocyte/macrophage-like cells; they found that miR-222 is over-expressed after TPA exposure, while its expression is negative without TPA-induction. This suggests that miR-222 is involved in tumor progression. Tsai et al. (29)studied the miRNA profile in human embryonic stem (hES) cells, and found that miR-222 was down-regulated in several hES cell lines, indicating its involvement in the regulation of basic stem cell functions. He et al. (13) found that numerous miRNAs are up-regulated in papillary thyroid carcinoma (PTC) tumors compared with unaffected thyroid tissue; miR-222 was the most over-expressed (10.9-fold) in PTC tumors, and they concluded that miR-222 over-expression and regulation of KIT are involved in PTC pathogenesis.

Some miRNAs that were differentially expressed in gonadotropin-producing pituitary adenomas shared other characteristics. Like miR-222, miR-523 was over-expressed in gonadotropin-producing pituitary adenoma compared to normal pituitary (average 6.2-fold up-regulation) and is also over-expressed in placenta (11). miR-503, the most down-regulated gene (39.8-fold) in gonadotropin-producing pituitary adenoma, is located on the X chromosome, and is over-expressed in placenta and testis and down-regulated in brain and thyroid. The X chromosomal position and the over-expression in sex organs indicate that these miRNAs may be closely related with sex hormones—and sex hormone metabolism disorders may be a cause of gonadotropin pituitary adenomas. The similarities between miR-10b, miR-222, miR-523, and miR-503 suggest that their synergistic actions may be critical in the occurrence of gonadotropin-secreting pituitary adenomas.

We found miR-31 was down-regulated 22.7-fold in gonadotropin-secreting pituitary adenomas and 21.5-fold in non-functioning pituitary adenomas compared to normal pituitaries. Located on the No.9 chromosome, miR-31 is reportedly expressed in the breast , colorectal, prostate and others human tissues (9,12,18,24). Mitra et al. (21) found that in ovarian Cancer-associated fibroblasts, miR-31 was down-regulated compared with normal or tumor-adjacent fibroblasts. Zhao et al. (32), on the contrary, found that miR-31 was up-regulated expressed between Cancer-associated fibroblasts and normal fibroblasts in breast cancer. Furthermore, Valastyan et al. (30) found miR-31 can impede metastasis of breast cancer as a metastasis suppressor gene, and miR-31 levels in primary human breast tumors were inversely associated with the possibility of the distant metastases. In our study, miR-31 is down-regulated significantly, although we cannot confirm that it can be treated as an anti-oncogene, at least it plays an important role in the tumorigenesis processes of pituitary adenomas.

Further studies are needed to experimentally verify the results of the present study and to correlate them with pituitary adenomas oncogenesis. To screen for true pituitary adenoma oncogenes, cultured pituitary adenomas cell can be used to determine if miRNA knockdown will trigger activation of caspases and lead to increased apoptotic cell death.

REFERENCES

1. Amaral FC, Torres N, Saggioro F, Neder L, Machado HR, Silva WA Jr, Moreira AC, Castro M: MicroRNAs differentially expressed in ACTH-secreting pituitary tumors. J Clin Endocrinol Metab 94:320-323, 2009

2. Bentwich I, Avniel A, Karov Y, Aharonov R, Gilad S, Barad O, Barzilai A, Einat P, Einav U, Meiri E, Sharon E, Spector Y, Bentwich Z: Identification of hundreds of conserved and nonconserved human microRNAs. Nat Genet 37:766-770, 2005
3. Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC: miR-15a and miR-16-1 down-regulation in pituitary adenomas. J Cell Physiol 204: 280-285, 2005
4. Bottoni A, Zatelli MC, Ferracin M, Tagliati F, Piccin D, Vignali C, Calin GA, Negrini M, Croce CM, Degli Uberti EC: Identification of differentially expressed microRNAs by microarray: A possible role for microRNA genes in pituitary adenomas. J Cell Physiol 210:370-377, 2007
5. Butz H, Likó I, Czirják S, Igaz P, Korbonits M, Rácz K, Patócs A: MicroRNA profile indicates downregulation of the TGFβ pathway in sporadic non-functioning pituitary adenomas. Pituitary 14:112-124, 2011
6. Chile T, Corrêa-Giannella ML, Fortes MA, Bronstein MD, Cunha-Neto MB, Giannella-Neto D, Giorgi RR: Expression of CRABP1, GRP, and RERG mRNA in clinically non-functioning and functioning pituitary adenomas. J Endocrinol Invest 34: 214-218, 2011
7. Davoren PA, McNeill RE, Lowery AJ, Kerin MJ, Miller N: Identification of suitable endogenous control genes for microRNA gene expression analysis in human breast cancer. BMC Mol Biol 9:76-80, 2008
8. de Oliveira JC, Brassesco MS, Scrideli CA, Tone LG, Narendran A: MicroRNA expression and activity in pediatric acute lymphoblastic leukemia (ALL). Pediatr Blood Cancer 59: 599-604, 2012
9. Fuse M, Kojima S, Enokida H, Chiyomaru T, Yoshino H, Nohata N, Kinoshita T, Sakamoto S, Naya Y, Nakagawa M, Ichikawa T, Seki N: Tumor suppressive microRNAs (miR-222 and miR-31) regulate molecular pathways based on microRNA expression signature in prostate cancer. J Hum Genet 57:691-699, 2012
10. Gabriely G, Teplyuk NM, Krichevsky AM: Context effect: MicroRNA-10b in cancer cell proliferation, spread and death. Autophagy 7:1384-1386, 2011
11. Garzon R, Pichiorri F, Palumbo T, Iuliano R, Cimmino A, Aqeilan R, Volinia S, Bhatt D, Alder H, Marcucci G, Calin GA, Liu CG, Bloomfield CD,Andreeff M, Croce CM: MicroRNA fingerprints during human megakaryocytopoiesis. Proc Natl Acad Sci USA 103: 5078-5083, 2006
12. Guan P, Yin Z, Li X, Wu W, Zhou B: Meta-analysis of human lung cancer microRNA expression profiling studies comparingcancer tissues with normal tissues. J Exp Clin Cancer Res 31:54, 2012
13. He H, Jazdzewski K, Li W, Liyanarachchi S, Nagy R, Volinia S, Calin GA, Liu CG, Franssila K, Suster S, Kloos RT, Croce CM, de la Chapelle A: The role of microRNA genes in papillary thyroid carcinoma. Proc Natl Acad Sci USA 102: 19075-19080, 2005
14. Kasashima K, Nakamura Y, Kozu T: Altered expression profiles of microRNAs during TPA-induced differentiation of HL-60 cells. Biochem Biophys Res Commun 322: 403-410, 2004
15. Krichevsky AM, King KS, Donahue CP, Khrapko K, Kosik KS: A microRNA array reveals extensive regulation of microRNAs during brain development. RNA 9:1274-1281, 2003
16. Larysz D, Zebracka-Gala J, Rudnik A, Hasse-Lazar K, Kowalska M, Jarząb M, Król A, Szpak-Ulczok S, Bażowski P, Jarząb B. Expression of genes FOLR1, BAG1 and LAPTM4B in functioning and non-functioningpituitary adenomas. Folia Neuropathol 50:277-286, 2012
17. Liu N, Chen NY, Cui RX, Li WF, Li Y, Wei RR, Zhang MY, Sun Y, Huang BJ, Chen M, He QM, Jiang N, Chen L, Cho WC, Yun JP, Zeng J, Liu LZ, Li L,Guo Y, Wang HY, Ma J: Prognostic value of a microRNA signature in nasopharyngeal carcinoma: A microRNA expression analysis. Lancet Oncol 13:633-641, 2012
18. Lu Z, Ye Y, Jiao D, Qiao J, Cui S, Liu Z: MiR-155 and miR-31 are differentially expressed in breast cancer patients and are correlated with the estrogen receptor and progesterone receptor status. Oncol Lett 4:1027-1032, 2012
19. Mao ZG, He DS, Zhou J, Yao B, Xiao WW, Chen CH, Zhu YH, Wang HJ: Differential expression of microRNAs in GH-secreting pituitary adenomas. Diagn Pathol 7:79-82, 2010
20. Melmed S: Pathogenesis of pituitary tumors. Nat Rev Endocrinol 7:257-266, 2011
21. Mitra AK, Zillhardt M, Hua Y, Tiwari P, Murmann AE, Peter ME, Lengyel E: MicroRNAs reprogram normal fibroblasts into cancer-associated fibroblasts in ovarian cancer. Cancer Discov 2:1100-1108, 2012
22. Nugent M, Miller N, Kerin MJ: MicroRNAs in colorectal cancer: Function, dysregulation and potential as novel biomarkers. Eur J Surg Oncol 37:649-654, 2011
23. Rostad S: Pituitary adenoma pathogenesis: An update. Curr Opin Endocrinol Diabetes Obes 19:322-327, 2012
24. Schee K, Boye K, Abrahamsen TW, Fodstad Ø, Flatmark K: Clinical relevance of microRNA miR-21, miR-31, miR-92a, miR-101, miR-106a and miR-145 in colorectalcancer. BMC Cancer 12:505, 2012
25. Shi X, Tao B, He H, Sun Q, Fan C, Bian L, Zhao W, Lu YC: MicroRNAs-based network: A novel therapeutic agent in pituitary adenoma. Med Hypotheses 78:380-384, 2012
26. Sivapragasam M, Rotondo F, Lloyd RV, Scheithauer BW, Cusimano M, Syro LV, Kovacs K: MicroRNAs in the human pituitary. Endocr Pathol 22:134-143, 2011
27. Smirnova L, Grafe A, Seiler A, Schumacher S, Nitsch R, Wulczyn FG: Regulation of miRNA expression during neural cell specification. Eur J Neurosci 21:1469-1477, 2005
28. Stilling G, Sun Z, Zhang S, Jin L, Righi A, Kovācs G, Korbonits M, Scheithauer BW, Kovacs K, Lloyd RV: MicroRNA expression in ACTH producing pituitary tumors: Up-regulation of microRNA-122 and -493 in pituitary carcinomas. Endocrine 38:67-75, 2010
29. Tsai ZY, Singh S, Yu SL, Kao LP, Chen BZ, Ho BC, Yang PC, Li SS: Identification of microRNAs regulated by activin A in human embryonic stem cells. J Cell Biochem 109:93-102, 2010

30. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szász AM, Wang ZC, Brock JE, Richardson AL, Weinberg RA: A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell 137:1032-1046, 2009
31. Wu Q, Wang C, Lu Z, Guo L, Ge Q: Analysis of serum genome-wide microRNAs for breast cancer detection. Clin Chim Acta 413:1058-1065, 2012
32. Zhao L, Sun Y, Hou Y, Peng Q, Wang L, Luo H, Tang X, Zeng Z, Liu M: MiRNA expression analysis of cancer-associated fibroblasts and normal fibroblasts in breast cancer. Int J Biochem Cell Biol 44:2051-2059, 2012