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ORIGINAL ARTICLE

MicroRNA expression in ACTH-producing pituitary tumors: up-regulation of microRNA-122 and -493 in pituitary carcinomas

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Abstract MicroRNAs (miRNAs) are involved in cell proliferation, differentiation, and apoptosis, and can function as tumor suppressor genes or oncogenes. The expression of miRNAs in pituitary carcinomas has not been previously examined. We used miRNA profiling with 1,145 probes to study miRNA expression in normal anterior pituitary (6 cases), adrenocorticotropin (ACTH)-producing adenomas (8 cases), and ACTH-producing pituitary carcinomas (two cases). Real-time RT-PCR and in situ hybridization were used to confirm and independently validate miRNAs that were significantly up-regulated or down-regulated between the pituitary tissues. There were more miRNAs up- (188) or down-regulated (160) between adenomas and normal pituitaries compared to carcinomas and normal pituitaries (92 up- and 91 down-regulated) or between carcinomas and adenomas (46 up- and 52 down-regulated). Both real-time RT-PCR and in situ hybridization showed significant up-regulation of miRNA-122 between pituitary carcinomas and adenomas. MiRNA-493 was also up-regulated in carcinomas compared to ACTH adenomas. Analysis of genes that miRNA-493 interacts with included LGALS3 and RUNX2 (http://microrna.sanger.ac.uk) both of which have been shown to have roles in pituitary tumor cell growth. These results provide information about marker miRNAs that may lead to further insights into the regulation of pituitary tumor growth and development.

Keywords MicroRNA · Pituitary adenoma · Pituitary carcinoma · In situ hybridization

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Introduction

MicroRNAs (miRNAs) are non-coding 20–24 nucleotide RNAs that function in gene regulation [1–3]. MiRNAs are excised from 60–110 nucleotide fold back RNA precursor moleculars and are involved in critical biological processes such as development, differentiation, apoptosis, and proliferation [1–5]. They interact with target mRNAs at specific sites to induce cleavage of the message or to inhibit translation. MiRNAs are transcribed from intergenic or intronic DNA as large precursors which then undergo successive enzymatic processing by Drosha, Pasha, and Dicer into their mature 20–24 nucleotide, double-stranded

RNA. MiRNA duplexes are incorporated into the RNA-induced silencing complex, and the mature miRNA strand is preferentially retained. A number of malignancies have been associated with miRNA signatures, and they have been found to be dysregulated in malignancies functioning as oncogenes or tumor suppressor genes [1–7].

MiRNAs have been detected in normal pituitaries and in pituitary adenomas [8–10]. MiRNA 15a and 16-1 have been found to be down-regulated in pituitary adenomas compared to normal pituitary tissues [8, 9]. Amaral et al. examined a series of normal pituitary tissues and ACTH-secreting pituitary tumors for miRNA expression and reported under expression of miRNA-145, -21, -141, let-7a, -150, -15a, -16, and -143 in ACTH-secreting pituitary tumors compared to normal pituitary tissues [10].

Pituitary carcinomas, defined by the development of metastatic disease, are rare [11, 12]. MiRNA expression has not been previously examined in pituitary carcinomas. Altered expression of specific miRNAs in pituitary carcinomas may provide insight into the pathogenesis of the tumors and may also provide useful diagnostic information to distinguish adenomas from pituitary carcinomas before they metastasize. We analyzed a series of normal pituitaries, ACTH adenomas, and ACTH carcinomas to examine the expression of specific miRNAs in these tissues. The findings were validated for two specific miRNAs by real-time RT-PCR and by in situ hybridization.

Materials and methods

Tissues

Samples used for the analyses included frozen tissues of 8 ACTH-producing adenomas and 2 ACTH-producing pituitary carcinomas. Seven normal pituitary tissues were obtained from post-mortem patients autopsied within 4 h after death. The 8 ACTH adenomas included 6 functioning tumors and one type 1 and one type 2 silent ACTH adenomas. The metastatic ACTH carcinomas included a liver metastasis from a 66-year-old female (Case 1) and a periparotid lymph node metastasis from a 31-year-old female (Case 2). Both patients with ACTH carcinomas died of their disease. Samples were frozen within 30 min after surgery. Autopsy samples were frozen within 6 h post-mortem. The quality of extracted RNA was examined for each case before using in experiments.

An additional number of adenomas (4 PRL, 4 GH, 4 gonadotrophs, and 4 null cell tumors) were used for real-time RT-PCR analysis. In situ hybridization included formalin-fixed paraffin-embedded specimens from 4 normal pituitaries, 4 prolactin (PRL), 3 growth hormone (GH), 4 gonadotroph, 4 null cells, and 7 ACTH adenomas as well as 8 pituitary carcinomas (7 ACTH and 1 PRL). All of the pituitary tumors were characterized by immunohistochemistry as previously reported [13]. IRB permission was obtained for the study.

Tissue collection and RNA extraction

Tumors and normal pituitary tissues were frozen in liquid nitrogen and stored at −70°C until used. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) as previously reported [13, 14]. The concentration and quality of each RNA sample was determined using the A260/A280 spectrophotometric reading. RNA was store at −70°C until used. Agilent tracings were performed to analyze the quality of the RNA before using for miRNA profiling.

MicroRNA profiling on high throughput microarray platform

Illumina's miRNA expression profiling panel was used in this study (12-sample per Chip Universal Bead Array). The panel contains 1,145 probes which targeted 687 unique miRNAs (171 redundant probes) selected from miRBase database (Release 12.0) and 287 miRNAs from Illumina re-sequencing (41 miRNAs) and the literature (246 miRNAs) [15].

Microarray data analysis

The microarray raw intensity data were imported into Illumina Beadstudio version 3.1 (gene expression module version 3.4) to obtain signal intensity and detection *P* value for each microRNA transcript. The data without background subtraction and normalization were first obtained to assess the data quality. All samples passed quality assessment using average signal intensity, detected number of miRNAs, and signal-to-noise ratio. These data were then exported to R, the statistical analysis package (http://www.r-project.org/), for further processing. The exported data were log 2 transformed and normalized using function fastlo [16]. After the normalization, a pair of embedded universal controls and a pair of sample replicates across the two different bead chips were assessed for their reproducibility. One normal sample was excluded from further analysis as its overall profile from other normal samples as identified by unsupervised hierarchical clustering. Further review found the patient to have a history of hypothyroidism. In order to reduce background noise, we filtered out 163 probes with detection a *P* value >0.05 across all 16 samples. Unsupervised clustering with the remaining 982 probes was conducted to assess the overall expression profiles of the samples. The analysis of variance (ANOVA)

model was used to detect differentially expressed miRNAs in the three types of tissues: carcinoma, adenoma, and normal anterior pituitary. Pair-wised contrasts were created to identify the differentially expressed miRNAs between carcinoma and normal pituitary, adenoma and normal pituitary, and carcinoma and adenoma. Multiple testing was adjusted using a step-up procedure implemented in Partek software, version 6.4., Partek Inc., St. Louis, MO, USA. A fold change cut-off was determined by looking at log2 ratio distribution for all miRNAs from each comparison. Differentially expressed miRNAs (P value <0.05) were separated into three groups according to log2 ratio >1, 2, or 3 standard deviations (SD). MiRNAs with P value <0.05 and fold change >3, which is roughly >2 SD, were the central focus for the study. Heat maps were created for these top changed miRNAs using the Dchip software package (http://biosun1.harvard.edu/complab/dchip/) [17] where the distance metric used was 1 minus the correlation, and the linkage method was centroid.

Real-time RT-PCR

MiRNAs-122 and -493 were selected from the RNA profiling studies for further analysis. Mature miRNA expression levels were measured using the TaqMan MicroRNA Assay kit (Applied Biosystems, Foster City, CA, USA). Briefly, total RNA of 10 ng for miRNA-493 and 60 ng for miRNA-122 were reverse transcribed in a total volume of 15 µl using the TaqMan MicroRNA Reverse Transcription kit according to the manufacturer's instruction. 1.33 µl of each RT reaction product was amplified for qPCR in 20 µl total containing 10 µl of TaqMan 2× Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems). Each PCR reaction was performed in triplicate on 384-well plates using the ABI 7900HT Fast Real-Time PCR system for 40 cycles. Small nucleolar RNA, RNU48, was co-amplified as an endogenous control. Three independent experiments with three replicates were performed for each sample.

Calculation of relative expression level

Threshold cycle (C_t) values were defined as the cycle number at which the fluorescence exceeded a fixed threshold value above an automatically set baseline. RNU 48 was selected as the endogenous control. C_t values of each target miRNA transcript were normalized against the C_t value of RNU 48. The relative change of miRNA expression of miRNA-122 and miRNA-493 in each sample compared to a normal pituitary were then calculated using the $\Delta\Delta C_t$ method. Three replicates for each miRNA and control were performed and averaged. The standard error of the mean was determined for each sample.

In situ hybridization

In situ hybridization to detect miRNA-122 and -493 was performed using formalin-fixed paraffin-embedded tissues including 4 normal pituitaries, 21 pituitary adenomas (3 GH, 4 PRL, 6 ACTH, 4 gonadotrophs, and 4 null cells), and 8 pituitary carcinomas. Probes for in situ hybridization were purchased from Exiqon (Denmark and Woburn, MA, USA) and consisted of locked nucleic acid (LNA) oligonucleotide probes labeled at both the 5′′ and 3′ ends with digoxigenin. In situ hybridization was performed as previously reported [14]. Briefly, it involved microwave pretreatment in a 700-W oven in 10 mM citric acid pH 6.0 for 12 min followed by cooling at room temperature for 20 min. Tissues were then digested in 25 mg/ml proteinase K solution at 37°C for 10 min. Hybridization was performed at 55°C overnight in a humidified chamber using 200 nM of probe. Slides were washed in 2× sodium chloride–sodium citrate (SSC) at 50°C for 15 min, followed by washing in 0.5× SSC at 50°C for 15 min. Slides were then rinsed in Tris-buffer saline (TBS, pH 7.5) buffer (buffer A) and incubated with anti-digoxygenin–alkaline phosphatase labeled antibody (Roche) at 1:200 dilution in buffer A with 1% normal swine serum and 0.3% Triton X-100 for 3 h. After washing in buffer A and then buffer C (TBS, pH 9.5), the slides were incubated with nitroblue-tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, at pH 9.5 in buffer C and developed between 30 min to 3 h. The slides were counterstained with 0.1% Nuclear Fast Red for 3 min. The negative control consisted of substituting a scrambled probe (Exiqon) at the same concentration as the hybridization probe. As a second control, slides were treated with RNAse A (250 µg/ml from Sigma Chemical, ST. Louis, MO) before hybridization. Both the scramble probe and RNAse pretreatment resulted in no hybridization signal. The hybridization signals were scored as 0 or negative, 1+ (weak) staining, 2+ (moderate) staining, and 3+ (strong) staining. A mean score ± the standard error of the mean was obtained for each group by adding the score of each case in the group and dividing by the number of cases.

Statistical analysis

The student t test was used to analyze for significant differences in the real-time RT-PCR and for in situ hybridization analyses. A P value of <0.05 with the two-tail test was considered significant.

Results

Microarray data results

Overall miRNA expression profiling

Unsupervised clustering was used to evaluate overall miRNA expression patterns among the three different types of tissues. Normal pituitary gland tissues and adenomas had very different miRNA expression patterns and they formed two major clusters. However, the two carcinomas seemed indistinguishable from the adenomas.

The number of significantly changed miRNAs between the study groups of normal pituitary, ACTH adenomas, and ACTH carcinomas are shown in Table 1. Comparisons of ACTH adenomas versus normal pituitaries showed the largest number of changed genes. The changed miRNAs between ACTH adenomas and normal anterior pituitary tissues as well as between ACTH carcinomas and adenomas having a P value <0.05 and the different cut-off ranges are shown in the Supplementary Data Table 1. The highest number of miRNAs to be up-regulated or down-regulated were present in the comparison between adenomas and normal pituitaries. The least number of changes in miRNAs were evident in the carcinoma versus adenoma groups (Table 1).

MiRNAs with the greatest changes (P values <0.05 and fold change >3) are shown in the heat maps in Figs. 1, 2, and 3. The comparison of carcinomas to normal anterior pituitary tissues had the greatest number of changed miRNAs with a P value <0.05 and fold change >3 (Supplementary Data Table 1).

Two miRNAs were selected for further analyses based on their degree of changes detected by miRNA profiling. MiRNA-122 showed a 14.30-fold difference between carcinomas and adenomas and a 10.793-fold change between

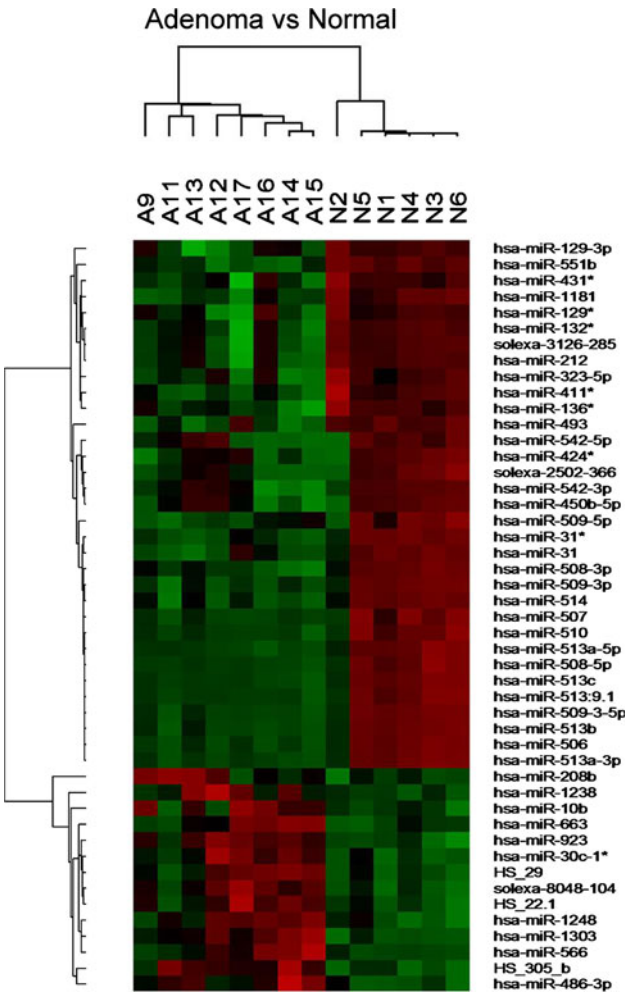

Fig. 1 Heat map showing highly changed miRNAs between ACTH adenomas and normal pituitaries ($P < 0.05$ and FC > 3) where samples are in the *columns* and miRNAs are in the *rows*. *A* adenoma, *N* normal pituitary. The color scale illustrates the relative expression level of miRNA across all samples with *red* for higher and *green* for lower expression

Table 1 Number of significantly changed miRNAs between different comparisons

Comparison	Direction	$P < 0.05$	$P < 0.05$ and FC > 1 SD	$P < 0.05$ and FC > 2 SD	$P < 0.05$ and FC > 3 SD	$P < 0.05$ and FC > 3
Adenoma vs. Normal	Up	188	74	14	3	14
	Down	160	85	33	18	33
Carcinoma vs. Normal	Up	92	59	14	4	18
	Down	91	62	29	18	33
Carcinoma vs. Adenoma	Up	46	26	11	2	4
	Down	52	37	18	5	11

SD standard deviation, *FC* fold change

Significantly changed miRNAs between ACTH adenomas and normal anterior pituitary tissues, between the ACTH carcinoma and normal pituitary gland, and between ACTH carcinomas and ACTH adenoma (a P value <0.05 and different cut-offs of fold change). Up represents up-regulation and down represents down-regulation in the specific group. The entries in the *last column* represents genes with $P < 0.05$ and FC < 3

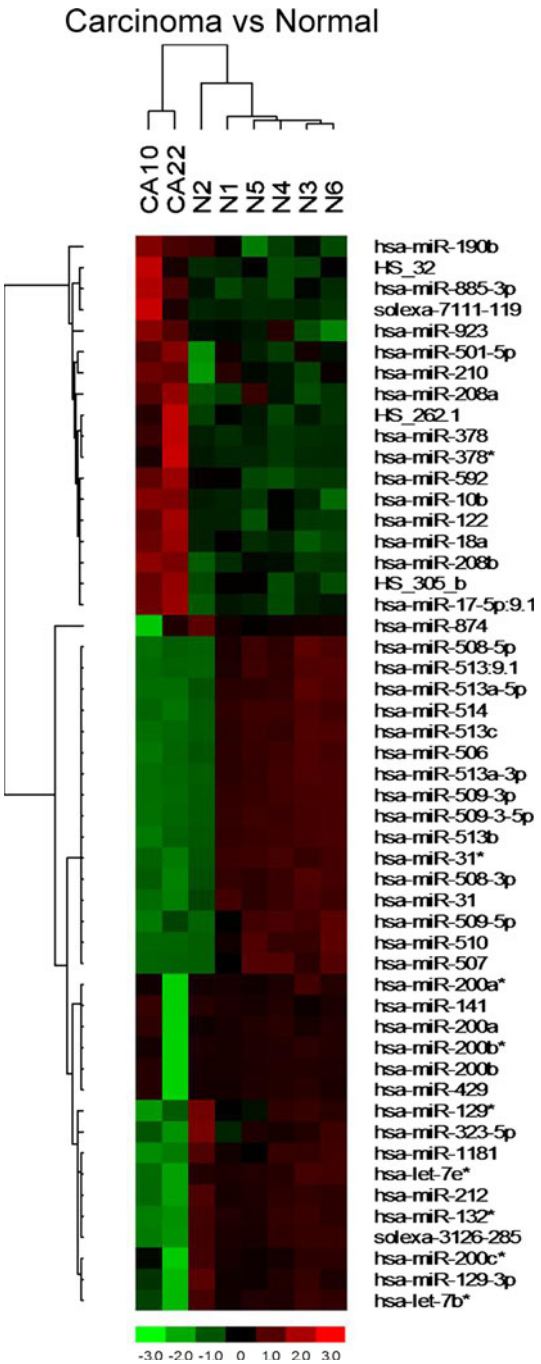

Fig. 2 Heat map of highly changed miRNAs between ACTH carcinomas and normal pituitaries ($P < 0.05$ and FC > 3) where samples are in the *columns* and miRNAs are in the *rows*. *CA* carcinoma, *N* normal pituitary. The color scale illustrates the relative expression level of miRNA across all samples with *red* for higher and *green* for lower expression

carcinoma and normal pituitaries. MiRNA 493 showed a 3.465-fold change between carcinoma and adenoma as well as a −4.571-fold change between adenomas and normal pituitary (Supplementary Data Table 1).

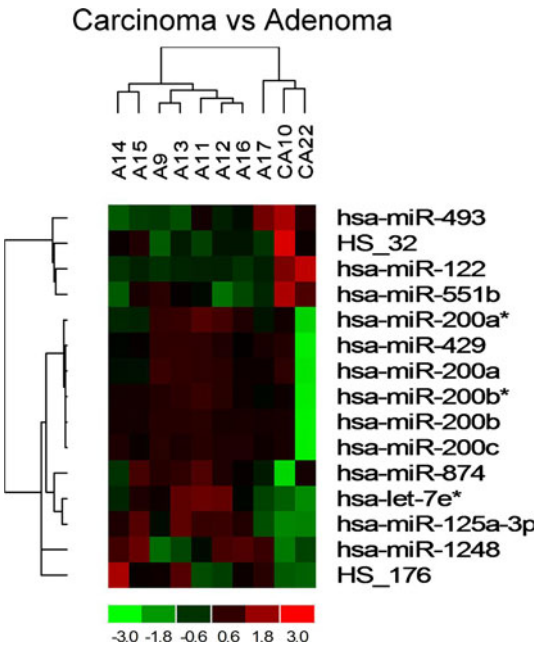

Fig. 3 Heat map showing highly changed miRNAs between ACTH carcinomas and ACTH adenomas ($P < 0.05$ and FC > 3) where samples are in the *columns* and miRNAs are in the *rows*. *CA* carcinoma, *A* adenoma. The color scale illustrates the relative expression of miRNAs across all samples with *red* for higher and *green* for lower expression

Real-time RT-PCR

Real-time RT-PCR analysis of normal pituitary and ACTH adenomas used the same samples analyzed in the RNA profiling analysis. In addition to the two carcinoma samples used in the RNA profiling analysis, two additional carcinoma samples from Case 2 (liver metastasis and brain metastasis) were examined, and new cases of GH, PRL, gonadotroph, and null cell adenomas were also analyzed ($n = 15$). The carcinomas had the highest levels of overexpression of miRNA-122 compared to all groups of adenomas and normal pituitaries (Fig. 4). The level of expression of miRNA-122 was significantly higher in the ACTH carcinomas compared to ACTH adenomas ($P < 0.001$) or other adenoma subtypes (Fig. 4).

Real-time RT-PCR of miRNA-493 showed significantly higher levels of this miRNA in the two carcinomas compared to the ACTH, null cell, and gonadotroph adenomas (Fig. 5A, B). The amount of miRNA-493 in PRL and GH adenomas was significantly greater than in other adenoma types.

In situ hybridization

In situ hybridization analysis with LNA probes showed localization of miRNA-122 and -493 in the paraffin sections (Figs. 6a–f). The specificity of the reaction was

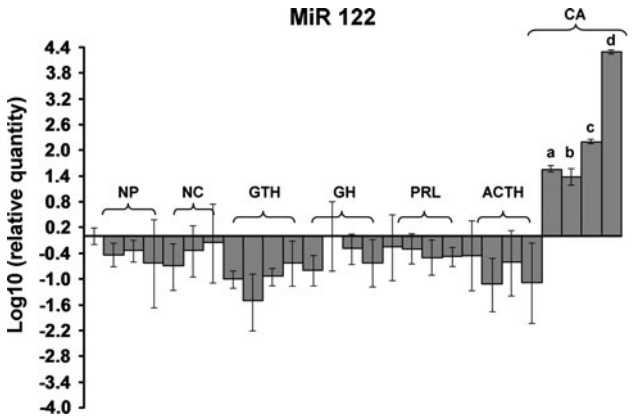

Fig. 4 Expression levels of microRNA-122 (miR 122) in normal pituitary, pituitary adenomas, and pituitary carcinomas based on real-time RT-PCR analyses. Three replicates were used for each sample. The log 10 and standard error of the mean are shown for each sample. *NI* normal pituitary, *NC* null cell adenoma, *GTH* gonadotroph adenoma, *GH* growth hormone adenoma, *PRL* prolactin adenoma, *ACTH* ACTH adenoma, *CA-ACTH* carcinoma. Sample a from CA—liver metastasis from Case 1 (sample 1). Sample b from CA—brain metastasis from Case 2. Sample c—periparotid lymph node metastasis from Case 2. Sample d from CA—liver metastasis from Case 2. The ACTH carcinomas were significantly different from the ACTH adenomas ($P < 0.001$), PRL adenomas ($P < 0.001$), GH adenomas ($P < 0.001$), GTH adenomas ($P < 0.001$), and null cell adenomas ($P < 0.001$)

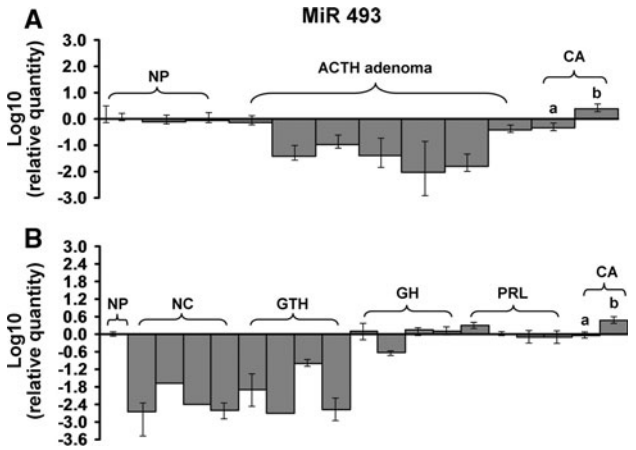

Fig. 5 Expression levels of miRNA-493 (miR 493) in normal pituitary ACTH adenomas and pituitary carcinomas after real-time RT-PCR analysis. Three replicas were used for each sample. The log 10 and standard deviations are shown for each sample. **A** *NP* normal pituitary—ACTH tumors, *CA* ACTH carcinomas from two cases; *a*—1 liver metastasis from Case 1; *b*—brain metastasis from Case 2. **B** *NP* normal pituitary, *NC* null cell adenomas, *GTH* gonadotroph adenoma, *GH* growth hormone adenoma, *PRL* prolactin adenoma, *CA* ACTH carcinomas from two cases; *a*—case 1 liver metastasis; *b*—case 2 brain metastasis. The ACTH carcinomas were significantly different from the ACTH adenomas ($P < 0.05$), the null cell adenomas ($P < 0.001$), and the gonadotroph adenomas ($P < 0.05$)

shown by RNase digestion before ISH (not shown) and by using a scrambled LNA probe instead of the specific miRNAs (Figs. 6a, b). There was strong positive expression of miRNA-122 in ACTH pituitary carcinomas (Fig. 6A; Table 2). A total of 22 adenomas, 4 normal pituitary, and 8 pituitary carcinomas (7 ACTH and 1 PRL) were analyzed by ISH. These tumor cases were not used in the RNA profiling or real-time RT-PCR experiments. Normal anterior pituitary showed diffuse weak expression for miRNA-122 (Fig. 6c). MiRNA-493 showed variable expression in the normal pituitary with focal cells showing moderately strong (2+) expression (Fig. 6d). ISH with miRNA-493 showed stronger expression in the ACTH carcinomas compared to the ACTH and other adenomas (Table 2; Fig. 6e, f). There was generally weak expression of miRNA-493 in gonadotroph, null cell, PRL, and GH adenomas compared to the carcinomas (Table 2).

Discussion

In the present study, miRNA expression profiling in normal pituitaries, ACTH adenomas, and ACTH carcinomas showed a number of miRNAs to be differentially expressed in different pituitary tissues. Comparison of normal anterior pituitaries and adenomas showed the largest number of genes up- or down-regulated in the study, while comparison of carcinomas and adenomas showed the least number of genes up- or down-regulated. We observed that miRNA-122 was significantly up-regulated in the two cases of pituitary carcinomas compared to normal pituitaries and ACTH adenomas. One of the ACTH carcinomas had metastatic carcinoma in three separate sites. When the three sites were analyzed, they all showed up-regulation of miRNA-122 by real-time RT-PCR. In situ hybridization with independent samples also corroborated the up-regulation of miRNA-122 in pituitary carcinomas. The one PRL carcinoma examined by in situ hybridization also showed similar up-regulation or miRNA-122 as did the ACTH carcinomas.

MiRNA-122 is highly expressed in liver tissue and is thought to be a liver-specific miRNA [18–21] where it functions as a tumor suppressor because it is down-regulated in hepatocellular carcinomas. We examined a case of normal liver tissue by real-time RT-PCR and found high levels of miRNA-122 similar to those in the pituitary carcinomas. Although we examined two cases of pituitary carcinomas metastatic to the liver, we concluded that the miRNA-122 was from the pituitary, since very little normal liver was present in the specimens and in situ hybridization showed a strong hybridization signal for miRNA-122 in both the pituitary cells and liver cells.

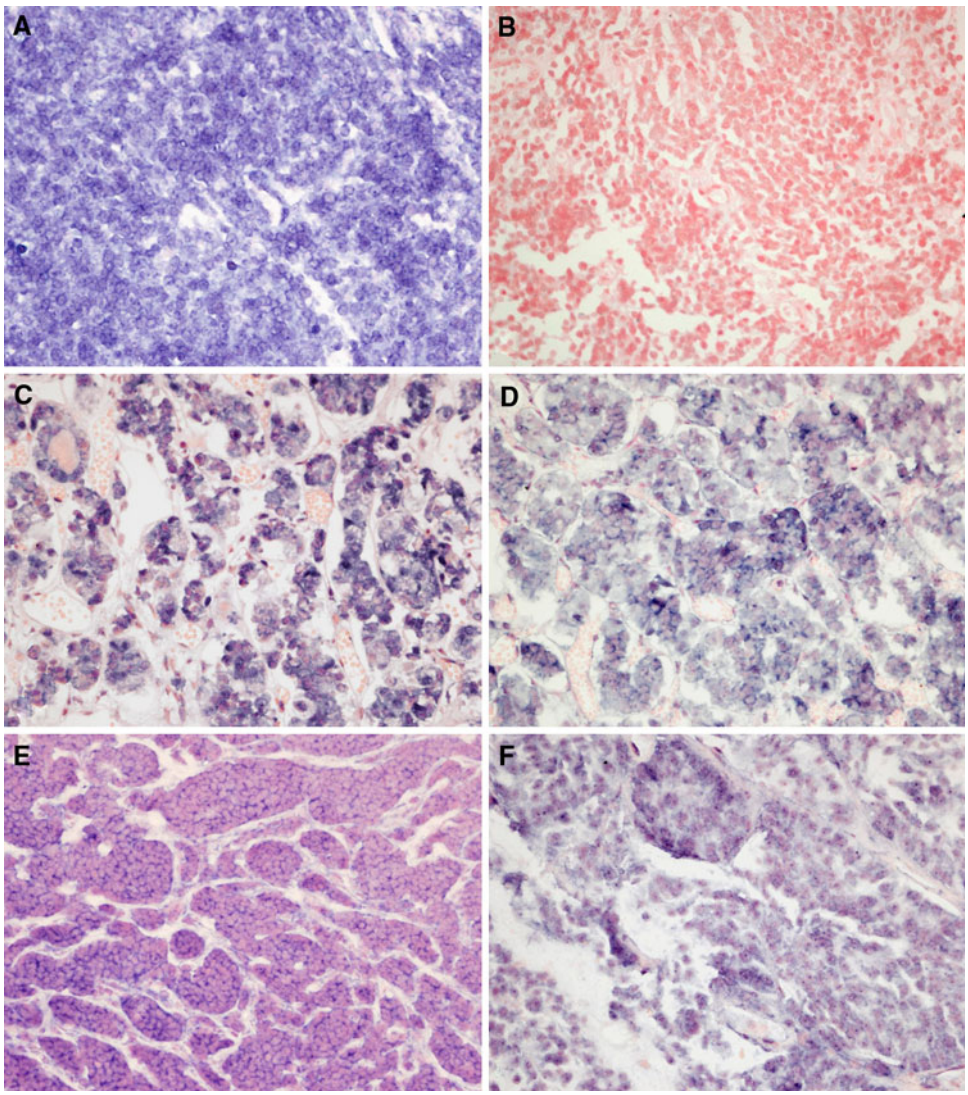

Fig. 6 In situ hybridization with locked nucleic acid probes for miRNA-122 and -493. **a** Pituitary carcinomas showing strong staining (3+) for miRNA-122. **b** Same case as in **a** with a scramble control LNA probe showed no hybridization signal. **c** Normal pituitary showing diffuse but weak (1+) hybridization signal for miRNA-122. **d** Normal pituitary hybridization with miRNA-493 probe showing focal (2+) staining in a few cells. **e** ACTH carcinoma showing a moderate hybridization signal (2+) with miRNA-493. **f** ACTH adenoma showing weak staining (1+) by in situ hybridization with miRNA-493

Table 2 In situ hybridization analysis of microRNA-122 and -493 expressions in normal pituitaries, pituitary adenomas, and carcinomas

Diagnosis	*N*	In situ hybridization score MiRNA 122	In situ hybridization score MiRNA 493
Normal pituitary	4	1.2 ± 0.5	1.5 ± 0.5
ACTH adenomas	7	1.0 ± 0.3**	0.86 ± 0.3**
PRL adenomas	4	0.67 ± 0.3*	0.75 ± 0.3**
GH adenomas	3	0.67 ± 0.3*	0.67 ± 0.3*
Gonadotroph adenomas	4	1.5 ± 0.3*	1.5 ± 0.3*
Null cell adenomas	4	1.8 ± 0.5	1.3 ± 0.3*
Pituitary carcinomas	8	2.4 ± 0.2	2.3 ± 0.2

N number of cases in the group

In situ hybridization was performed as outlines in "Material and methods" section. The intensity of staining was graded as 0 negative, 1+ weakly positive, 2+ moderately positive, and 3+ strongly positive. Focal staining indicates <25% of the normal pituitary or tumor cells with positive staining. Results are expressed as mean score ± standard error of the mean

* $P < 0.05$; ** $P < 0.01$ compared to pituitary carcinoma

Kreutzfeldt et al. [22] reported that silencing of liver miRNA-122 led to increased expression of several hundred genes, which suggest that they are miRNA-122 target mRNAs including mRNAs normally repressed in hepatocytes [22]. Although miRNA-122 appears to be relatively liver-specific, recent studies have shown that infection of cell lines with adenovirus Ad-miR122 led to apoptosis and/or cell cycle arrests in various cell lines including those from liver, lung, and uterine cervix. These observations suggest that miRNA-122 has regulatory roles in different cell types [23]. A recent study using LNA-modified oligonucleotide (SPC 3649) complementary to miRNA-122 led to long-lasting suppression of hepatitis C viremia suggesting a clinical role for the use of this miRNA [24].

Our miRNA profiling results were confirmed by real-time RT-PCR and in situ hybridization. They support the observation that miRNA-122 was expressed in normal, benign, and malignant pituitary tissues. Earlier studies of normal pituitaries and ACTH adenomas [10] did not detect differential expression of miRNA-122. However, this miRNA was detected mainly in comparing ACTH carcinomas and adenomas in the miRNA profiling studies. This suggests that alteration of this miRNA may be associated with the development of malignant pituitary tumors.

MiRNA-493 was expressed in both normal and neoplastic pituitary tissues, although the levels were higher in the two ACTH carcinomas. Differences in the expression levels were significantly greater in pituitary carcinomas compared to ACTH adenomas. MiRNA-493 has not been studied extensively in human tissues but has been reported in aging mouse lungs [25] and it has been studied in some cell lines [26]. The possible functions of this miRNA are uncertain. The Sanger miRBase database suggests that miRNA-493 interacts with LGALS3 and RUNX2 genes (http://microrna.sanger.ac.uk) [27]. Our recent studies showed that the RUNX2 gene regulates the LGALS-3 gene. LGALS-3 gene is expressed mainly in ACTH and PRL pituitary tumors [13, 28–30]. Our previous studies also showed that galectin-3, the product of the LGALS-3 gene, had a role in regulating cell proliferation and apoptosis in pituitary cells. Thus, studies of the role of miRNA-493 in regulating proliferation and apoptosis in pituitary tumors may provide insights into the functions of this miRNA in pituitary cells.

Analyses of miRNA-122 and -493 expressions by in situ hybridization with LNA showed the feasibility of detecting specific miRNAs in formalin-fixed paraffin-embedded tissues [17]. Although there is probably significant loss of miRNAs during the processing of these tissues, the combined sensitivity of LNA probes, labeling with digoxigenin at the 3′ and 5′ ends, and sensitive in situ hybridization techniques indicate that this approach can be used to localize specific miRNAs in paraffin-embedded sections of pituitary and other tissues with optimal preservation of morphologic features [17, 31–33].

Only a limited number of studies on miRNA expression in pituitary tumors have been reported [8–10, 34, 35]. Zatelli and degli Uberti [34] noted that miRNA-15a and 16-1, which are down-regulated in pituitary adenomas compared to normal pituitaries, are located on chromosome 13q14 along with the tumor suppressor gene RB. Aggressive pituitary adenomas and carcinomas have a deletion in proximity to the RB gene [36, 37]. Earlier studies also showed that miRNA expression could differentiate non-functional microadenomas from macroadenomas [8, 34]. Qian et al. [35] reported that miRNA let-7 expression was inversely correlated with high mobility group A2 expression, and decreased expression of let-7 was present in some pituitary tumors and was correlated with high grade tumors [35]. The roles of miRNA-122 and -493 in pituitary function are unknown and will be investigated in future studies.

In summary, our findings of high levels of miRNA-122 expression in pituitary carcinomas compared to adenomas and normal pituitaries suggest that miRNA-122 may have a role in pituitary carcinoma development and may be useful in detecting differences between benign pituitary tumors and more aggressive pituitary tumors that may be more likely to recur after surgery. MiRNA-493 which binds to the LGALS3 and RUNX2 genes and which is up-regulated in ACTH carcinomas compared to adenomas raises questions about the role of this miRNA in regulating pituitary cell proliferation and tumor development.

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References

1. G.A. Cailn, C.M. Croce, Nat. Rev. Cancer **6**, 857–866 (2006) (Review)
2. A. Esquela-Kerscher, F.J. Slack, Nat. Rev. Cancer **6**, 259–269 (2006)
3. E. Barbarotto, T.D. Schmittgen, G.A. Calin, Int. J. Cancer **122**, 969–977 (2008)
4. O.A. Kent, J.T. Mendell, Oncogene **25**, 6188–6196 (2006)
5. W. Zhang, J.E. Dahlberg, W. Tam, Am. J. Pathol. **171**, 728–738 (2007)
6. T. Du, P.D. Zamore, Development **132**, 4645–4652 (2005)
7. L. He, J.M. Thomson, M.T. Hemann, E. Hernando-Monge, D. Mu, S. Goodson, S. Power, C. Cardon-Cardo, S.W. Lowe, G.J. Hannon, S.M. Hammon, Nature **435**, 828–833 (2005)
8. A. Bottoni, D. Piccin, F. Tagliati, A. Luchin, M.C. Zatelli, E.C. degli Uberti, J. Cell Physiol. **204**, 280–285 (2005)
9. A. Bottoni, M.C. Zatelli, M. Ferracin, F. Tagliati, D. Piccin, C. Vignali, G. Calin, J. Cell Physiol. **210**, 370–377 (2007)

10. F.C. Amaral, N. Torres, F. Saggioro, L. Neder, H.R. Machado, W.A. Silva Jr., A.C. Moreira, M. Castro, J. Clin. Endocrinol. Metab. **94**, 320–323 (2009)
11. B.W. Scheithauer, O. Kurtkaya-Yapicier, K.T. Kovacs, W.R. Young Jr., R.V. Lloyd, Neurosurgery **56**, 1066–1074 (2005)
12. N. Sanno, A. Teramoto, R.Y. Osamura, E. Horvath, K. Kovacs, R.V. Lloyd, B.W. Scheithauer, Neurosurg. Clin. N. Am. **14**, 25–39 (2003)
13. L. Jin, D. Riss, K. Ruebel, S. Kajita, B.W. Scheithauer, E. Horvath, K. Kovacs, R.V. Lloyd, Endocr. Pathol. **16**, 107–114 (2005)
14. K. Ruebel, A.A. Leontovich, G.A. Stilling, S. Zhang, A. Righi, L. Jin, R.V. Lloyd, Mod. Pathol. (2009) [Epub ahead of print]
15. E. Berezikov, F. Therneau, L.W. van Laake, I. Kondava, R. Bontrop, E. Cuppen, R. Plasterk, Nat. Genet. **38**, 1375–1377 (2006)
16. K.V. Ballman, D.E. Grill, A.L. Oberg, T. Thernau, Bioinformatics **20**, 2778–2786 (2004)
17. C. Li, W.H. Wong, Proc. Natl. Acad. Sci. USA **98**, 31–36 (2001)
18. M. Lagos-Quintana, R. Rauhut, A. Yalcin, J. Meyer, W. Lendeckel, T. Tuschl, Curr. Biol. **12**, 735–739 (2002)
19. X. Wu, S. Wu, L. Tong, T. Luan, L. Lin, S. Lu, W. Zhao, Q. Ma, H. Liu, Z. Zhong, Scand. J. Gastroenterol. **44**, 1332–1339 (2009)
20. S. Bai, M.W. Nasser, B. Wang, S.H. Hsu, J. Datta, H. Kutay, A. Yadav, G. Nuovo, P. Kumar, K. Ghoshal, J. Biol. Chem. **284**, 32015–32027 (2009)
21. M. Girard, E. Jacquemm, A. Munnich, S. Lyonnet, A. Henrion-Clande, Rev. J. Hepatol. **48**, 648–656 (2008)
22. J. Krutzfeldt, N. Rajewsky, R. Braich, K.G. Rajeev, T. Tuschl, M. Manoharan, M. Stoffel, Nature **438**, 685–689 (2005)
23. L. Ma, J. Liu, J. Shen, L. Liu, J. Wu, W. Li, J. Luo, Q. Chen, C. Qian, Cancer Biol. Ther. **20**, 9(7) (2010) [Epub ahead of print]
24. R.E. Lanford, E.S. Hildebrandt-Eriksen, A. Petri, R. Persson, M. Lindow, M.E. Munk, S. Kauppinen, H. Orum, Science **327**, 198–201 (2010)
25. A.E. Williams, M.M. Perry, S.A. Moschos, M.A. Lindsay, BMC Genomics **8**, 172 (2007)
26. Y.L. Shen, Y.G. Jiang, A.R. Greenlee, L.L. Zhou, L.H. Liu, Biomed. Environ. Sci. **22**, 14–21 (2009)
27. S. Griffith-Jones, R.J. Grocock, S. van Dongen, A. Bateman, A.J. Enright, Nucl.-Acids Res. **34**, D140–D144 (2006)
28. D. Riss, L. Jin, X. Qian, J. Bayliss, B.W. Scheithauer, W.F. Young Jr, S. Vidal, K. Kovacs, A. Raz, R.V. Lloyd, Cancer Res. **63**, 2251–2255 (2003)
29. K.H. Ruebel, L. Jin, X. Qian, B.W. Scheithauer, K. Kovacs, N. Nakamura, H. Zhang, A. Raz, R.V. Lloyd, Cancer Res. **65**, 1136–1140 (2005)
30. H.Y. Zhang, L. Jin, G.A. Stilling, K.H. Ruebel, K. Coonse, Y. Tanizaki, A. Raz, R.V. Lloyd, Endocrine **35**, 101–111 (2009)
31. P.T. Nelson, D.A. Baldwin, W.P. Kloosteman, S. Kauppinen, R. Plasterk, Z. Mourelatos, RNA **12**, 187–191 (2006)
32. R. Thomsen, P.S. Nielsen, T.H. Jensen, RNA **11**, 1745–1748 (2005)
33. G.J. Nuovo, Methods **44**, 39–46 (2008)
34. M.C. Zatelli, E.C. degli Uberti, Seminars in Reproductive Medicine **26**, 453–460 (2008)
35. Z.R. Qian, S.L. Asa, H. Siomi, M.C. Siomi, K. Yoshimoto, S. Yamada, E.L. Wang, M.M. Rahman, H. Inoue, M. Itakura, E. Kudo, T. Sano, Mod. Pathol. **22**, 431–441 (2009)
36. A.S. Bates, W.E. Farrell, E.J. Bichnell, A.M. McNicol, A.J. Talbot, J.C. Broome, C.W. Perrett, R.V. Thakker, R.N. Clayton, J. Clin. Endocrinol. Metab. **82**, 818–824 (1997)
37. X. Fan, A. Paetau, Y. Aalto, M. Valimaki, T. Sane, A. Poranen, J.S. Castresana, S. Knuutila, Genet. Cytogenet. **128**, 97–103 (2001)