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Differential expression profiling of microRNAs and their potential involvement in renal cell carcinoma pathogenesis

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Abstract

Objective: We seek to identify the differentially expressed miRNAs in the clear cell subtype (ccRCC) of kidney cancer.

Design and methods: We performed a miRNA microarray analysis to compare the miRNA expression levels between ccRCC tissues and their normal counterpart. The top dysregulated miRNAs were validated by quantitative RT-PCR analysis. Bioinformatics analysis was also performed.

Results: A total of 33 dysregulated miRNAs were identified in ccRCC, including 21 upregulated miRNAs and many of these miRNAs have been reported to be dysregulated in other malignancies and have a potential role in cancer pathogenesis. The miRNAs showed a significant correlation with reported chromosomal aberration sites. We also utilized target prediction algorithms to identify gene targets. Preliminary analyses showed these targets can be directly involved in RCC pathogenesis.

Conclusion: We identified miRNAs that are dysregulated in ccRCC and bioinformatics analysis suggests that these miRNAs may be involved in cancer pathogenesis and have the potential to be biomarkers.

Keywords: miRNA; Kidney cancer; Microarray; RT-PCR; Bioinformatics; microRNA; Tumor markers; Renal cell carcinoma

Introduction

Kidney cancer is a common urologic malignancy that accounts for about 3% of adult malignancies [1]. It causes about 90,000 deaths worldwide annually, with the clear cell histological subtype being the most common, representing about 75–80% of renal cell carcinoma (RCC). Apart from surgery, it is both chemotherapy and radiotherapy resistant. At present, biomarkers for early detection and follow-up of the disease are not available, accounting for late diagnosis and subsequent poor prognosis. Therefore, a more clear understanding of the pathogenesis of RCC is required for developing new target therapies and biomarkers that predict treatment efficacy.

A class of small noncoding RNAs, called microRNAs (miRNAs), is recently discovered and shown to regulate gene expression at the post-transcriptional level, by binding through partial sequence homology, to the 3′ untranslated region (3′ UTR) of mammalian target mRNAs and causing translational inhibition and/or mRNA degradation [2]. Mature miRNAs are 18–25 nucleotides long and are the result of sequential processing of primary transcripts (pri-miRNAs) mediated by a complex protein system that includes two RNase III enzymes, Drosha and Dicer, members of the Argonaute family and Pol II-dependent transcription [3]. The pre-miRNA precursor is cleaved by cytoplasmic RNase III endonuclease Dicer into a 22 nucleotide mature double stranded miRNA. The strand which serves as mature miRNA, is incorporated into the RNA-induced silencing complex (RISC) and drives the selection of target mRNAs containing antisense sequences, thus controlling target gene expression [4].

miRNAs have been identified as key regulators in many biological processes including development, differentiation,

Abbreviations: qRT-PCR, quantitative reverse transcription polymerase chain reaction; RCC, renal cell carcinoma; miRNA, microRNA.

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apoptosis and proliferation. Moreover, it has been shown that miRNAs are aberrantly expressed or mutated in cancers, suggesting that they may play a role as a novel class of oncogenes or tumor suppressor genes [5,6]. miRNAs have been shown to be differentially expressed in a variety of cancers, including prostate, lung, breast, colon and other malignancies [7]. Recent evidence has shown diverse clinical applications of miRNAs in cancer, for diagnosis, prognosis and predictive purposes [8].

In this study, we performed an miRNA microarray to analyze the miRNA expression profile in the clear cell subtype of RCC compared to normal counterparts. We also validated our microarray results by performing quantitative RT-PCR for the top dysregulated miRNAs. This was then followed by a bioinformatics analysis to examine their potential contribution to the pathogenesis of RCC, future clinical significance, and their correlation with known chromosomal aberration sites in RCC.

Material and methods

Specimen collection

Fresh kidney tissues were obtained from patients who underwent nephrectomy for RCC, following the Research Ethics Board approval of St. Michael's Hospital. Three specimen pairs were dissected from both cancer and adjacent normal kidney cortex tissue from the same patient. The specimens were collected in cryotubes and stored at −70 °C for future use. All diagnoses were histologically verified by a pathologist.

Total RNA extraction

Total RNA extraction was performed with the mirVana extraction kit, following the manufacturer's protocol (Ambion, Austin, TX). The quality of extracted RNA was assessed by electropherogram and gel analysis, and an RNA integrity number (RIN) was calculated for every case. Only cases with RIN > 7 were included (Fig. 1).

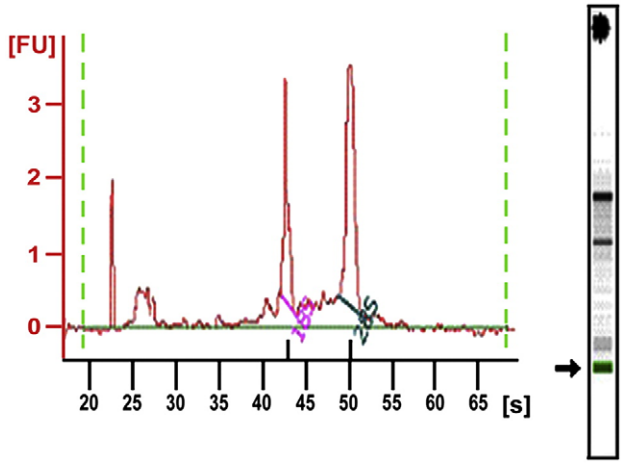

Fig. 1. Gel analysis of total RNA extraction from kidney tissue. The arrow highlights the band on the gel (right) that represents miRNA. The graph to the left of the gel is a bio-analysis graph of the RNA extraction sample, where 18S and 28S are used as our quality standards.

miRNA microarray

Microarray analysis was performed on 5 μg of total RNA from histologically confirmed cancer and adjacent normal tissues from the same patient, and was carried out using the μParaflo® microfluidic technology, as per the manufacturer's protocol (LC Sciences, Houston, TX). Hybridization was performed overnight on a microfluidic chip. On the microfluidic chip, each detection probe consisted of a chemically modified nucleotide coding segment complementary to target microRNA and a spacer segment of polyethylene glycol to extend the coding segment away from the substrate. The array covers all miRNA transcripts available in the latest version of the Sanger miRBase database (Release 11.0). Post-hybridization detection used fluorescence labeling with tag-specific Cy3 and Cy5 dyes. Hybridization images were collected using a GenePix 4000B laser scanner (Molecular Device, Sunnyvale, CA) and digitized using Array-Pro image analysis software (Media Cybernetics, Bethesda, MD). The data was analyzed by first subtracting the background and then normalizing the signals to balance the intensities of the Cy3 and Cy5 labeled transcripts so that differential expression ratios can be calculated. The ratio of the two sets of detected signals [$\log_2$ (cancer/normal)] and p values of the t test were calculated; differentially detected signals were those with a p value less than 0.01. Heat maps were generated for the differentially expressed miRNAs at the detectable signal. Dysregulated miRNAs were divided into high or low signal using a signal intensity value of 500 normalized signal units.

Quantitative RT-PCR

Quantitative miRNA RT-PCR was performed with the TaqMan microRNA assay® kit using the supplier's protocol (Applied Biosystems, Foster City, CA). The miRNA transcripts of three of the top dysregulated miRNAs (miR-199a*, miR-200c and miR-122) were first reverse-transcribed into cDNA using gene-specific miRNA qRT-PCR primer sets. This was followed by real-time PCR amplification for 40 cycles using ABI7500 Standard system and miRNA-specific probes (Applied Biosystems, Foster City, CA). Experiments were done in triplicates and an average was calculated. Expression values were normalized to a small nucleolar RNA, RNU44 (Applied Biosystems, Foster City, CA) which has been proven to have consistent expression levels in malignant and non-malignant tissue pairs [9]. Three pairs of kidney tumors and their adjacent normal kidney tissue were used for the RT-PCR analysis. Ct values were calculated by the ABI7500. ΔCt values were calculated using the Ct values of the miRNA probes and the RNU44 for each corresponding sample. ΔΔCt values are calculated using the ΔCt values of the normal tissue and the cancerous tissue for each miRNA probe. The fold change was expressed as a $\log_2$ value in keeping with the miRNA microarray output format.

Bioinformatics analysis

We used four programs to perform bioinformatics-based target prediction analysis. These are miRBase Targets V4 (http://microrna.sanger.ac.uk/targets/v4/), miRanda (http://www.

microrna.org/), TargetScan 4.0 (http://www.targetscan.org/), and PicTar predictions (http://pictar.bio.nyu.edu/). A positive prediction was only included if it was detected by at least two programs. miRNAs with the same mature sequences are treated as one miRNA. In silico analysis of miRNA expression in different malignancies was done by compiling a database of published dysregulated miRNAs in different cancer types (which included all publicly available published data at the time of analysis) [2,6,7,10–29]. This was used to compare the expression of miRNAs dysregulated in kidney cancer with other malignancies.

Chromosomal localization of dysregulated miRNAs were assigned from the miRBAse databases (Release 11.0) [30]. Four miRNAs, miR-19b, miR-26a, miR-29b and miR-199a* were excluded from this analysis since they were found to localize to more than one chromosomal site and in multiple clusters. We compiled the literature of the databases of chromosomal aberrations in kidney cancer from both the Mitelman database of chromosomal aberrations in cancer [31] and the Progenetix molecular cytogenetic database [32]. Chromosomal aberrations were defined as either gain or loss of the chromosomal material, and were calculated by subtraction in cases where both gain and loss were reported.

Table 1
miRNAs differentially expressed in clear cell RCC compared to normal counterparts.

miRNA	Log_2 (cancer/normal)	Dysregulation in cancer	*p* value
hsa-miR-122	4.48	Up	1.12E-02
hsa-miR-210	3.23	Up	6.55E-02
hsa-miR-101	2.46	Up	1.93E-02
hsa-miR-19b	2.04	Up	3.74E-02
hsa-miR-489	2.03	Up	9.92E-02
hsa-miR-20b	1.82	Up	2.36E-02
hsa-miR-340	1.51	Up	7.17E-02
hsa-miR-15a	1.41	Up	3.53E-02
hsa-miR-424	1.4	Up	4.71E-02
hsa-miR-106a	1.36	Up	1.46E-02
hsa-miR-20a	1.35	Up	2.05E-02
hsa-miR-106b	1.33	Up	9.27E-02
hsa-miR-17	1.27	Up	2.48E-02
hsa-miR-342-3p	1.23	Up	2.04E-02
hsa-miR-21	1.21	Up	2.79E-02
hsa-miR-126	0.96	Up	1.34E-02
hsa-miR-27a	0.89	Up	5.81E-02
hsa-miR-29c	0.79	Up	4.35E-02
hsa-miR-29b	0.77	Up	4.28E-02
hsa-miR-29a	0.61	Up	2.38E-02
hsa-miR-151-5p	0.44	Up	4.23E-02
hsa-miR-26a	−0.48	Down	2.24E-02
hsa-miR-191	−0.84	Down	1.87E-02
hsa-miR-378	−1.43	Down	6.87E-02
hsa-miR-532-5p	−1.9	Down	2.83E-02
hsa-miR-199a[a]	−1.9	Down	3.03E-02
hsa-miR-200b	−2.1	Down	3.59E-03
hsa-miR-182	−2.21	Down	5.15E-02
hsa-miR-1826	−2.24	Down	5.46E-02
hsa-miR-214	−2.47	Down	1.57E-02
hsa-miR-150	−2.76	Down	8.71E-02
hsa-miR-720	−3.47	Down	2.25E-02
hsa-miR-200c	−5.31	Down	1.44E-03

[a] Only microRNAs with high signal are showed here. For a complete list of all dysregulated miRNAs, please see Supplementary Table 1.

Results

miRNA microarray

We performed microarray analysis on three biological replicates. As summarized in Table 1 and Supplementary Table 1, we identified a total of 80 differentially expressed miRNAs in clear cell RCC compared to normal. These were classified as

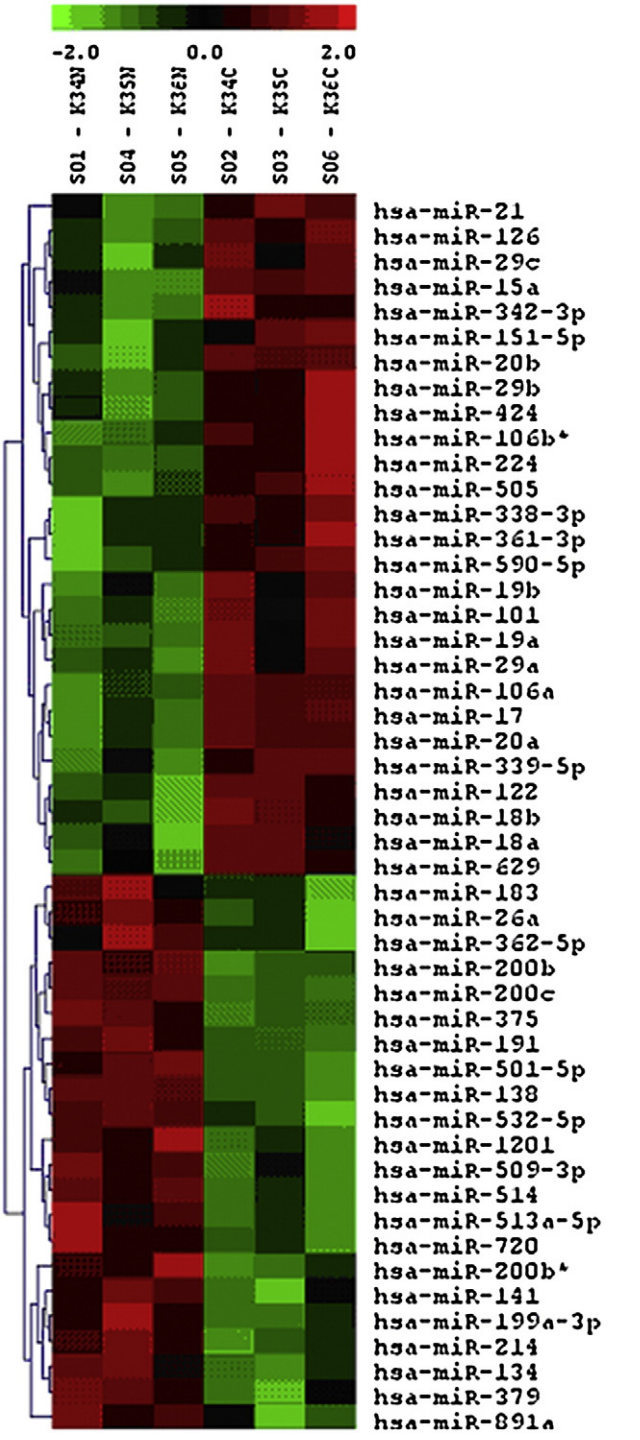

Fig. 2. Microarray heat map showing the statistically significant ($p<0.05$) dysregulated miRNAs in RCC.

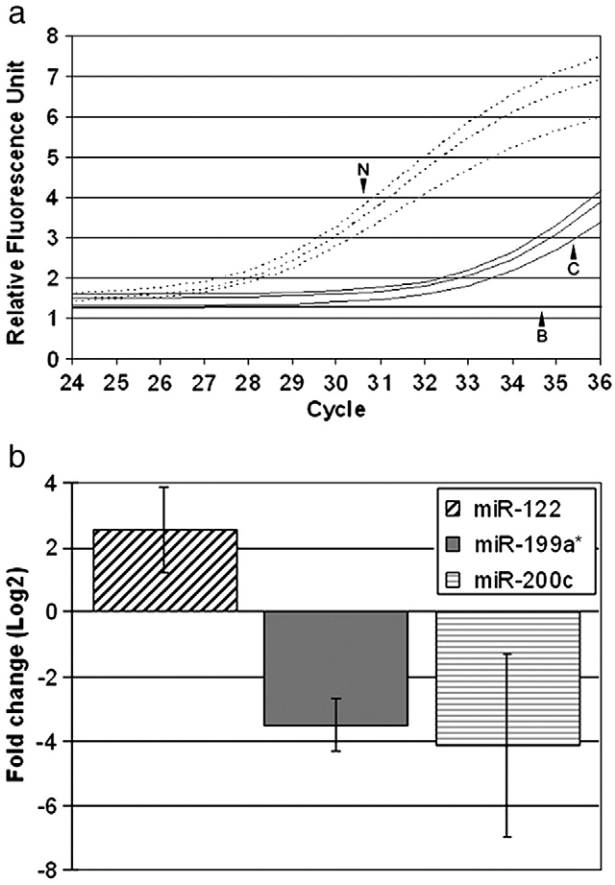

Fig. 3. Quantitative RT-PCR validation of the expression of miR-122, miR-199a* and miR-200c in kidney cancer. A. Graphic representation of the RT-PCR, where each line denotes the miRNA and type of tissue (cancer or normal). The graph indicates that the 3 miRNAs are differentially expressed in kidney cancer and are at comparable levels to those in Table 1. B. Bar graph of the log2 value of the fold change in cancer versus normal (normalized with the control, RNU44) of the 3 miRNAs. A positive value denotes an increase in cancer while a negative value denotes a decrease.

high and low signal groups (as described in the Materials and methods section). Among the high signal group, twenty one were upregulated and twelve downregulated. Fig. 2 shows a representative heat map of the statistically significant ($p<0.05$) results.

Experimental validation

We experimentally verified expression levels of three of the top dysregulated miRNAs, miR-122, miR-199a* and miR-200c on three different pairs of matching cancerous and normal tissues, with miRNA-specific probes using qRT-PCR analysis. As seen in Fig. 3, the $\log_2$ fold change values of the 3 miRNAs are comparable to the microarray results in Table 1.

Table 2
A partial list of the differentially expressed miRNAs in clear cell RCC and their association with other types of cancer.

miRNA	Log_2 (C/N)	Cancer type	Regulation	Reference
hsa-miR-106a	1.36	Colorectal	Up	28
		Lung	Up	30
		Lung (small cell)	Up	15
		Lymphoma	Up	17
		Pancreas	Up	28
		Prostate	Up	28
hsa-miR-191	−0.84	CLL	Down	12
		Breast	Up	18
		Colorectal	Up	28
		Lung	Up	28
		Pancreas	Up	28
		Pituitary Adenoma	Up	11
		Prostate	Up	28
		Stomach	Up	28
hsa-miR-20a	1.35	CLL	Down	12
		Colorectal	Up	28
		Lung (small cell)	Up	15
		Lymphoma	Up	17
		Pancreas	Up	28
		Prostate	Up	28
hsa-miR-21	1.21	CLL	Up	9
			Up	12
		Brain (glioblastoma)	Up	13
		Breast	Up	18
			Up	28
		Cervix	Up	20
		Colorectal	Up	28
		head and neck	Up	26
		Liver	Up	21
		Lung	Up	28
			Up	30
		Pancreas	Up	28
		Pancreas (adenocarcinoma)	Up	19
		Prostate	Up	28
		Stomach	Up	28
		Thyroid (PTC)	Up	16
		Uterus (ULM)	Up	29
hsa-miR-210	3.23	Breast	Up	18
			Up	28
		CLL	Down	12
		HCC	Up	21
		Lung	Up	28
			Up	30
		Pancreas	Up	25
		Prostate	Up	24
hsa-miR-214	−2.47	Colorectal	Up	28
		HCC	Down	14
		Lung	Up	30
		Pancreas	Up	28
		Prostate	Up	28
		Stomach	Up	28
		Thyroid (CRC cell line)	Down	10
hsa-miR-26a	−0.48	Lung	Down	30
		Pancreas	Up	28
		Pituitary Adenoma	Up	11
		Prostate	Down	24
			Up	28
		Thyroid (anaplastic carcinoma)	Down	27
		Thyroid (PTC)	Up	16

Note to Table 2:
Abbreviations: CLL—chronic lymphocytic leukemia, HCC—hepatocellular carcinoma, AML—acute myeloid leukemia, PTC—papillary carcinoma of the thyroid, ULM—uterine leiomyoma.

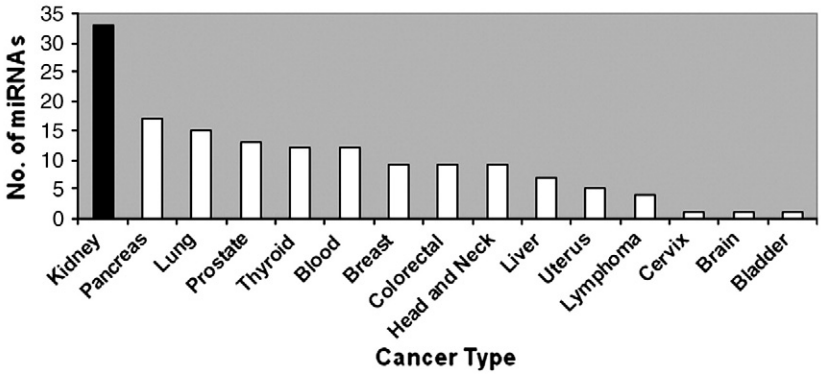

Fig. 4. Overlapped dysregulated miRNAs between kidney cancer and other types of cancer. There are overlaps between miRNAs that are differentially expressed in kidney cancer and those in other cancer types.

Expression of RCC dysregulated miRNAs in other malignancies

We compared experimental results from 23 published studies on miRNA differential expression in different cancers to our results [2,6,7,10–29]. 28 of the 33 dysregulated miRNAs identified in our study also have an association with a variety of other cancer types, including prostate and lung to brain and bladder cancers. A partial list is presented in Table 2 and the full list is shown in Supplementary Table 2.

A variable degree of overlap of dysregulated miRNAs was found in different cancer types ranging from 3% in brain, cervix and bladder cancers to about 52% (17 miRNAs) in pancreatic cancer (Fig. 4). Interestingly, no significant overlap was found between RCC (clear cell type) and other urological malignancies. In prostate cancer, only 13 common dysregulated miRNAs where found and, only one miRNA identified to be common with bladder cancer. Unique miRNAs that were not found to be dysregulated in other malignancies were miR-1826, miR-20b, miR-489, miR-532 and miR-720. These results further confirm earlier reports of the potential use of a "tissue specific" expression signature to distinguish different types of malignancies. "Commonly" dysregulated miRNAs in many cancers include miR-106a, miR-191, miR-21, miR-210 and miR-20a. These miRNAs and many others have been shown to regulate a number of cancer-related genes controlling cell cycle regulation, apoptosis, angiogenesis and cell migration through a variety of ways (some are discussed below) [33]. It should be noted, however, that comparison between different cancers needs to be verified by a more standardized approach that utilizes the same technique and method of quantification.

Target prediction analysis

In order to understand the potential involvement of miRNAs in the pathogenesis of RCC, we identified potential targets of the 33 dysregulated miRNAs using four different programs, as discussed in the Materials and methods section. As shown in

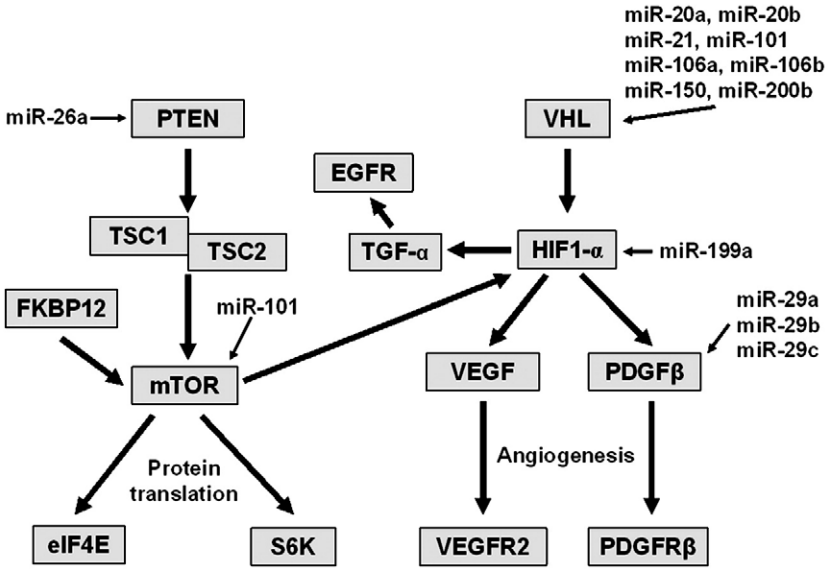

Fig. 5. RCC pathway and target mRNA prediction analysis. This kidney cancer pathway shows the different genes involved and the miRNAs that target them. 5 of the genes had differentially expressed miRNAs in RCC that target them.

Fig. 5, many of the genes that are known to be involved in RCC were detected as potential targets. The VHL and PDGFB genes have more than one miRNA that target them, showing that miRNAs also tightly regulate expression of genes at pathway and interaction level.

Chromosomal distribution of RCC dysregulated miRNAs

The chromosomal locations of the 33 differentially expressed miRNAs were mapped according to Release 11.0 of the miRBAse database [30] and compared to databases of chromosomal aberrations in kidney cancer (see Materials and methods section) (Fig. 6).

Twenty one out of 33 miRNAs were mapped to areas of reported chromosomal aberrations in RCC, 62% of them at gain sites and 38% at chromosomal loss sites. The 33 differentially expressed miRNAs were distributed among 15 chromosomes, with the largest miRNA clusters located on consistently reported sites of chromosomes 7, X and 1. There is a significant correlation between miRNA upregulation and chromosomal gain sites. For instance, 11 miRNAs that were upregulated were also located at chromosomal gain regions, compared to only 3 miRNAs that were downregulated and consistently reported at loss sites. Of importance is the 3p21.3 site, expressing a largely deleted 3p arm. A commonly dysregulated miRNA, miR-191 is located at this 3p21.3 highly consistent reported site, being associated with VHL regions. Another commonly dysregulated miRNA, miR-106a was reported to be located at chromosome Xq26.2 with a correspondence between miR-106a dysregulation and reported chromosomal aberration (Fig. 6). The reported

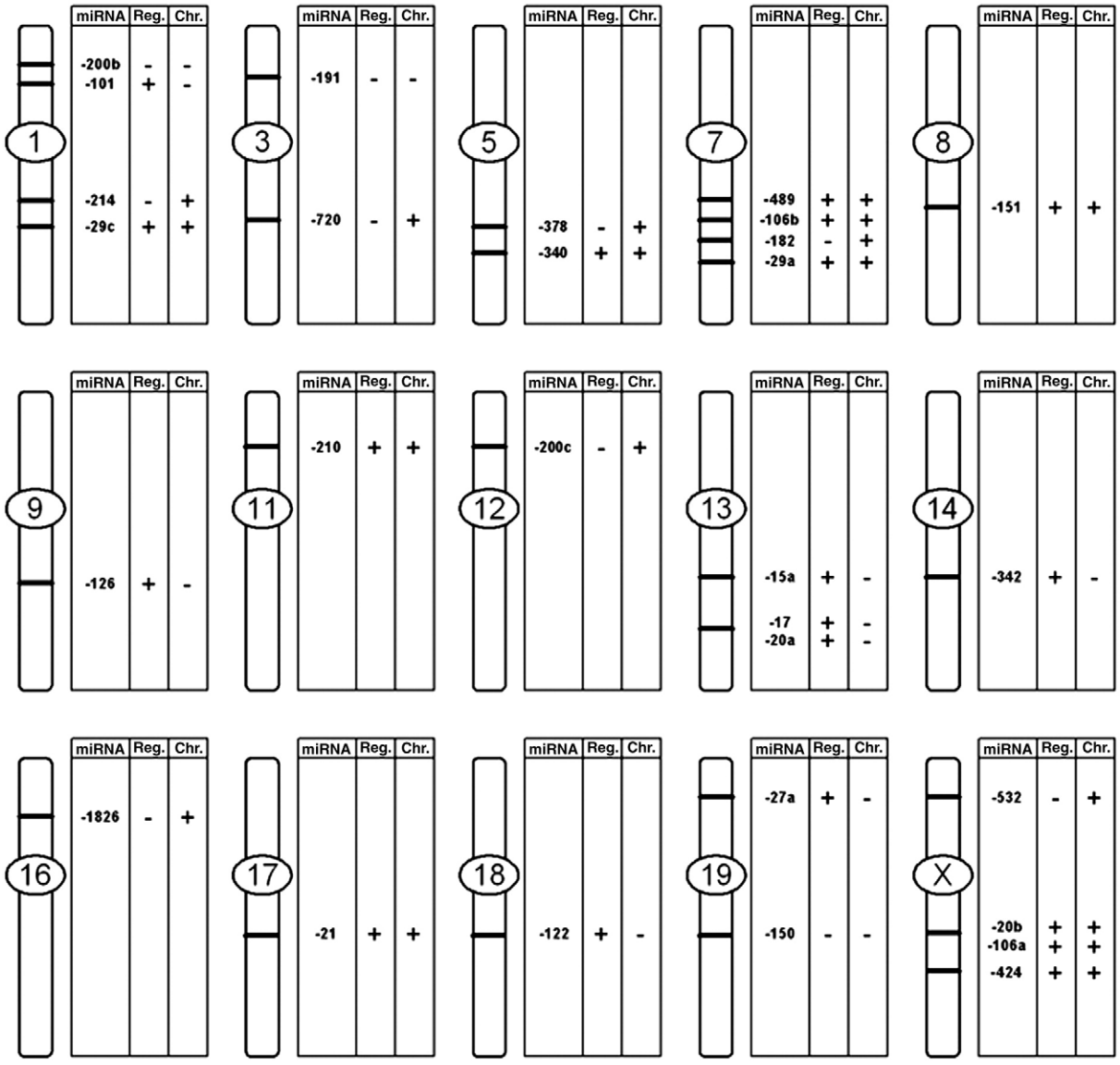

Fig. 6. Chromosomal locations of the differentially expressed miRNAs in kidney cancer and their correlation with chromosome aberrations. miRNA dysregulation (Reg.) represents the differential expression of the miRNA in kidney cancer. Chromosomal Aberration (Chr.) indicates whether the miRNA localizes to chromosomal regions with a gain (+) or with a loss (−). (Note: miR-19b, miR-26a, miR-29b and miR-199a were excluded from this analysis since they appeared to localize to more than 1 specific chromosomal site).

Table 3
Hypoxia-inducible miRNAs that are dysregulated in clear cell RCC.

miRNA	Fold Change in kidney cancer
hsa-miR-210	9.36
hsa-miR-106a	2.57
hsa-miR-21	2.32
hsa-miR-27a	1.85
hsa-miR-26a	0.718

chromosome 5q gain could elucidate the rich vascular nature of the tumor due to PECAM1 (Platelet-endothelial cell adhesion molecule-1) gene induction [34]. We speculate that the dysregulation of the miRNAs in this location could have a potential role for this induction. These results demonstrate that at least some miRNA differential expression can be attributed to gross chromosomal changes, but this is not the only mechanism that controls miRNA expression in cancer.

Hypoxia-inducible miRNAs

The link between hypoxia and kidney cancer is well documented in the literature. Mutations of the VHL gene lead to stabilization of hypoxia induced factors (HIFs) with subsequent turn on of proliferation signals. miRNAs, on the other hand, are reported to be affected by hypoxia. We compared our results to previously reported lists of hypoxia-inducible miRNAs [35]. As shown in Table 3, a total of five miRNAs from our experiment were presented as hypoxia-inducible, all being significantly dysregulated ($p<0.05$) in kidney cancer.

Discussion

In this report, we established miRNA expression profiling in clear cell RCC with qRT-PCR validation. This represents an initial step towards a better understanding of the involvement of miRNAs in RCC pathogenesis and opens the venue for new biomarker discovery and targeted therapy options. The miRNAs identified by microarray analysis and presented in Table 1 show about 64% of them with a significant upregulation ($p<0.05$), and the remainder being downregulated ($p<0.05$), a pattern consistent with miRNA expression profiles in other cancers [2,7,12,13]. Respectively, miR-122, miR-210 and miR-101 showed the highest upregulation levels, whereas miR-200c, miR-720 and miR-150 were the most downregulated (Table 1).

Out of the highly upregulated miRNAs, miR-210 was identified as being hypoxia-inducible (Table 3) and was also previously documented to be upregulated in other cancers that included breast, lung, prostate and liver (Table 2). Further analysis is needed to investigate the potential involvement of this miRNA in a "common" pathway of cancer development. Similar to our result, and also based on a miRNA microarray analysis, Camps C. et al, [36] identified and validated that in a shortage of oxygen levels, miR-210 had the most significant change. They and others showed that this was mediated through the Hypoxia Inducible Factor-1 alpha (HIF-1α)/Von Hippel Lindau (VHL) tumor suppressor system [36–38].

The tumor suppressor VHL gene is well documented to be inactivated in kidney cancer. It localizes to chromosome 3p25.3 where it acts to prevent tumor growth and is involved in regulating cellular signaling by hypoxia [39]. Hence, its loss will lead to a downstream signaling cascade of events that trigger hypoxia and cause an increase in cellular proliferation leading to cancer progression. HIF-1α is one of the key regulators of hypoxia response and transcription factors, allowing for the regulation of many genes and maintaining steady cell survival under low oxygen levels [35]. Using ChIP (Chromatin Immunoprecipitation) with a HIF-1α antibody, Kulshreshtha R. et al [35]), showed that HIF is directly recruited on the promoters of miR-210 and miR-26a under hypoxia, suggesting that hypoxia is involved in miRNA changes in cancer. miR-210 could also link hypoxia and cell cycle regulation in cancer [40].

Four out of the five hypoxia-inducible miRNAs in Table 3 were found to be upregulated. Extending these results further, one would expect low oxygen levels and signaling due to hypoxic conditions in cancer [40]. This implies that these upregulated miRNAs are induced in low oxygen levels due to tumor growth to inhibit the translation of their target gene, and hence have downstream biological impacts on cell survival and/or proliferation [41]. Based on our target prediction analysis, some of the hypoxia-inducible miRNAs could be implicated in RCC (Fig. 5). For instance, the VHL gene is targeted by miR-106a and miR-21. Downstream of VHL, miR-199a* which we show here as being downregulated in RCC usually targets HIF-1α to suppress the hypoxic effect.

The pro-apoptotic gene BAK1 is a target of miR-26a. miR-26a, as well as miR-210, is documented to have anti-apoptotic effects [35]. In a study by Wong CF, et al, [42] it was shown that miR-26a overexpression targets the histone methyltransferase, enhancer of Zeste homolog 2 (Ezh2). Normally, Ezh2 suppresses skeletal muscle differentiation. Thus, this presents an additional role for miR-26a in promoting cellular differentiation.

It has been also reported that, miR-21 is commonly upregulated in solid tumors of the lung, breast, stomach, prostate, colon, brain, head and neck, esophagus and pancreas, and here we show kidney. It is directly involved as an oncogene through a mechanism that involves translational repression of the tumor suppressor Programmed Cell Death 4 (PDCD4) gene by promoting cell transformation [43,44]. Moreover, as an oncogenic miRNA, mir-21 has a role not only in tumor growth but also in invasion and tumor metastasis by targeting and regulating genes like tropomyosin 1 (TPM1), which is another tumor suppressor gene that is involved in cell migration [45,46].

The Akt pathway is implicated in cancer since it causes apoptosis signaling inhibition, and it induces protein synthesis. The tumor suppressor PTEN can lead to the activation of the Akt pathway [47]. It is also implicated in the RCC pathway, and based on our target mRNA prediction we show that miR-26a can target PTEN and possibly block its translation leading to downstream signaling effects that lead to initiation of protein translation, mediated through the mTOR signaling pathway (Fig. 5).

Other miRNAs from Table 1 with known biological roles include miR-20a, miR-29c, miR-126, and miR-424. miR-20a

was identified as being upregulated in this study (Table 1) and it also has an anti-apoptotic role as shown in a study by Sylvestre Y. et al. [48] where overexpression of miR-20a decreased apoptosis in a prostate cancer cell line, while its inhibition led to an increase in cell death. It does so by modulating the translation of the E2F2 and E2F3 mRNAs via binding sites in their 3′-UTR. miR-29c, another upregulated miRNA has been shown to target genes that encode extracellular matrix proteins, including multiple collagens associated with tumorigenic invasiveness and thus most likely, metastasis [49]. Finally, miR-424 was shown to have an interesting role in controlling the monocyte/macrophage differentiation program [50].

We identified five miRNAs—miR-1826, miR-20b, miR-489, miR-532 and miR-720 that seem to be uniquely dysregulated in RCC but not in other cancer types (Table 3). A study in goats and sheep identified miR-720 expression with a possible role in skin and hair follicle growth and function [51].

In a recent study, Gottardo et al [1] performed miRNA microarray profiling in kidney and bladder cancers, where they identified four miRNAs—miR-28, miR-185, miR-7-2 and let-7f-2, to be significantly upregulated (and none as down-regulated) in kidney cancer with a 1.2 fold change cut-off. None of these miRNAs was identified in our analysis. Accountability for the differences observed could be attributed to the different experimental techniques and conditions used. For instance, we focused on the clear cell type of kidney cancer (RCC) compared to a mixture of histological types used in Gottardo et al. We also used kidney cortex as a normal control since kidney tumors, specifically of the clear cell type RCC arise from the kidney proximal renal tubular epithelium. The presence of cancer-specific miRNA signatures is not unprecedented. In another interesting study, Sun et al. [52], showed the presence of five kidney-specific miRNAs—miR-192, miR-194, miR-204, miR-215 and miR-216, which are expressed at much lower levels in other tissues. These miRNAs were found to have high sequence homology that is conserved among species. In addition, highly conserved transcription factor binding sites were found in the upstream sequence of these miRNAs. One of these is a highly conserved binding site for the proto-oncogene *ets-1*, which is a gene known to be essential for normal development of mammalian kidneys and maintenance of glomerular integrity [53]. Moreover, DNA sequences encoding for some of these miRNAs are located in chromosomal regions associated with kidney cancer [52]. Lastly, we also validated the expression of three of the top dysregulated miRNAs—miR-199a*, miR-122 and miR-200c by qRT-PCR, which is considered a gold standard technique, on three different specimens from those used in the microarray analysis.

In conclusion, we represent a differential expression profile for miRNAs in kidney cancer. We also provided evidence that link the biological role of miRNAs to cancer and showed that miRNAs can undertake a variety of mechanisms by which they can affect protein translation by regulating transcription factors and eventually alter processes like apoptosis/cell cycle regulation and cell migration. Further target validation analyses are needed for better understanding of miRNA function.

Acknowledgment

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.clinbiochem.2009.07.020.

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