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miR-210 Is a Prognostic Marker in Clear Cell Renal Cell Carcinoma

Sara Samaan,[*†] Heba W.Z. Khella,[*†] Andrew Girgis,[*†] Andreas Scorilas,[‡] Evi Lianidou,[§] Manal Gabril,[¶] Sergey N. Krylov,[‖**] Michael Jewett,[††‡‡] Georg A. Bjarnason,[¶¶] Hala El-said,[‖‖] and George M. Yousef[*†§§]

From the Department of Laboratory Medicine, and the Keenan Research Centre for Biomedical Science,† St. Michael's Hospital, Toronto, Ontario, Canada; the Departments of Biochemistry and Molecular Biology‡ and Chemistry,§ University of Athens, Athens, Greece; the Department of Pathology,¶ London Health Sciences Center and Western University, London, Ontario, Canada; the Department of Chemistry,‖ and Centre for Research on Biomolecular Interactions,** York University, Toronto, Ontario, Canada; the Division of Urologic Oncology,†† Princess Margaret Hospital, University Health Network, Toronto, Ontario, Canada; the Departments of Surgery‡‡ and Laboratory Medicine and Pathobiology,§§ University of Toronto, Toronto, Ontario, Canada; the Division of Medical Oncology and Hematology,¶¶ Sunnybrook Odette Cancer Center, Toronto, Ontario, Canada; and the Biochemistry Department,‖‖ The National Liver Institute, Menoufiya University, Al-Minufya, Egypt*

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Address correspondence to George M. Yousef, M.D., Ph.D., Department of Laboratory Medicine, St. Michael's Hospital, 30 Bond St., Toronto, ON M5B 1W8, Canada. E-mail: yousefg@smh.ca.

Accurate assessment of prognosis of clear cell renal cell carcinoma (ccRCC) is key in optimizing management plans to fit individual patient needs. miRNAs are short noncoding single-stranded RNAs that control the expression of target genes and may act as cancer biomarkers. We analyzed the expression of miR-210 in 276 cases of primary ccRCC and compared its expression in 40 pairs of adjacent normal and cancerous tissues. We assessed its expression in primary and metastatic tumors, in the common RCC subtypes, and the benign oncocytoma. The results were validated with an independent data set from The Cancer Genome Atlas. miR-210 was significantly overexpressed in ccRCC compared with normal kidney. miR-210^{+} patients had a statistically higher chance of disease recurrence [hazard ratio (HR), 1.82; $P = 0.018$] and shorter overall survival (HR, 2.46; $P = 0.014$). In multivariate analysis, miR-210 lost its statistically significant association with shorter disease-free survival and overall survival after adjusting for tumor size and tumor, node, metastasis stage. Papillary RCC showed comparable miR-210 overexpression, whereas decreased up-regulation was seen in chromophobe RCC and oncocytoma. A number of predicted targets that might be involved in carcinogenesis and aggressive tumor behavior were identified. miR-210 is a potential therapeutic target and independent marker of poor prognosis of ccRCC. *(J Mol Diagn 2015, 17: 136—144; http://dx.doi.org/10.1016/j.jmoldx.2014.10.005)*

Recent epidemiologic data have shown a rapid rise in the incidence of renal cell carcinoma (RCC), which is the most common type of kidney cancer in adults.[1,2] RCC encompasses a number of cancer subtypes, including clear cell, papillary, and chromophobe subtypes, which have distinct structural and cytogenetic characteristics.[3,4] The most common RCC subtype is clear cell (ccRCC), accounting for 80% of RCC cases. Recent reports have shown that the morphologic classification is not always accurate and that even within the same subtype subgroups have distinct biological behavior.[5,6]

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The 5-year survival rate varies greatly in ccRCC, ranging from 90% to 95% for tumors <4 cm, to approximately 60% in locally aggressive tumors that extend through the renal capsule or to the renal sinus. The 5-year survival for metastatic tumors drops significantly to approximately 5% to 15%. An accurate assessment of prognosis is essential in guiding the treatment decision for both primary and metastatic kidney cancer. In addition to surgical removal, other options for early-stage cancer include watchful waiting and percutaneous ablation of tumors. The treatment plans for metastatic RCC also rely on accurate assessment of prognosis.[7] Current prognostic assessment relies on clinical models.[8] There is still an urgent need for the discovery of molecular markers that can be used either alone or in combination with other clinical markers to better determine RCC prognosis.[9]

Previous studies have investigated the prognostic value of clinical markers such as tumor size and staging of tumor, node, metastasis (TNM) in ccRCC. Such investigations led to the subdivision of the T1 stage into two groups according to tumor size.[10,11] Furthermore, small renal masses can be classified as either progressive or nonprogressive according to their biological behavior.[12]

In the search for molecular markers miRNAs, which are short noncoding RNA nucleotides that regulate target expression post-transcriptionally, were reported to be dysregulated in RCC, pointing to their involvement in RCC pathogenesis.[13–15] Moreover, miRNAs have the potential to be useful diagnostic and prognostic markers and also potential therapeutic targets.[9,16,17] miRNAs are documented to be downstream effector molecules of the hypoxia inducible factor (HIF)-induced hypoxia response, but they may also be involved in non-HIF–mediated pathways. For example, miR-210 was implicated as a clinical marker because of its involvement with hypoxia in various cancers, including breast, lung, and pancreatic cancers.[16,18,19] In ccRCC, *VHL* gene mutations result in up-regulation of HIF-1 and HIF-2, with subsequent overexpression of miR-210.[20] Conversely, miR-210 was also shown to regulate HIF-1 protein in RCC and other target genes, affecting carcinogenesis-related processes such as cell migration and invasion, cell survival, mitochondrial metabolism, angiogenesis, apoptosis, and DNA damage repair.[19,21]

Here, we explored the clinical utility of miR-210 as a prognostic marker in ccRCC. We correlated its expression in ccRCC tissues with clinical markers, including tumor grade, stage, and size. We also investigated the correlation between miR-210 expression and disease-free and overall survival. We validated our findings in an independent set from The Cancer Genome Atlas (TCGA).

Materials and Methods

Patient Specimens

We analyzed a total of 284 cases of primary and metastatic kidney cancer tissues from patients with ccRCC. Tissues were collected from St. Michael's Hospital (Toronto, ON, Canada) and London Health Sciences Center (London, ON, Canada). Primary tumors were resected from therapy-naive patients. Diagnoses were confirmed by two independent genitourinary pathologists. Cancerous samples were taken from areas with no hemorrhage or necrosis, and six sections were mixed from the same tumor to compensate for tumor heterogeneity. Tumor classification and staging were established according to the 2002 TMN System and the 2004 World Health Organization classification. All procedures were performed according to approval from the Research Ethics Board of St. Michael's Hospital.

Samples from 40 pairs of normal/cancer tissues were extracted from fresh tissues obtained from the primary RCC from the same patient for comparing miRNA expression between normal and cancerous tissues. The expression of miR-210 was also compared between 264 cases of primary and 20 cases of metastatic ccRCC from different patients with the use of miRNA extracted from formalin-fixed tissues.

We also compared miR-210 expression between the subtypes of RCC by using fresh tissues obtained from 20 samples from each of ccRCC and papillary RCC, 16 samples of chromophobe RCC, and 15 oncocytoma specimens.

Total RNA Extraction

Six cores of pure tumor tissue were obtained from formalin-fixed, paraffin-embedded tissues and pooled for each specimen. Total RNA was extracted with miRNeasy (Qiagen, Mississauga, ON, Canada) according to the manufacturer's protocol, as described previously.[22] Total RNA concentrations were determined spectrophotometrically (NanoDrop 1000 Spectrophotometer; NanoDrop Technologies Inc., Wilmington, DE). Samples optimal for analysis were stored at −80°C.

RT-qPCR

Quantitative real-time RT-PCR (RT-qPCR) was used to measure miRNA expression with TaqMan MicroRNA Assays (Applied Biosystems, Foster City, CA) as described before.[23] miR-210–specific reverse transcription was performed with 5 ng of total RNA by using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) as recommended by the manufacturer. RT-qPCR was performed with the TaqMan microRNA Assay Kit on the Step One Plus Real-Time PCR System (Applied Biosystems). Thermal cycling conditions were consistent with the manufacturer's fast protocol, and all reactions were performed in triplicate. Gene expression analysis was performed with the comparative C_T ($2^{-\Delta\Delta C_T}$) method to calculate the relative quantification units of miR-210 in kidney tumors.

The comparative C_T ($2^{-\Delta\Delta C_T}$) method was used for performing relative quantification analysis.[24,25] The normalization of the miR-210 expression between different specimens

was implemented through *RNU44* and *RNU48* amplification by using one positive sample as a calibrator. With the use of the formula $\Delta C_T = C_T miR210 -$ geometrical mean of C_T *RNU*44 and C_T *RNU*48, we normalized the miR-210 expression of each tested sample to the *RNU44* and *RNU48* endogenous reference expression of the same sample. Consequently, by using the formula $\Delta\Delta C_T = \Delta C_T$, sample $- \Delta C_T$, calibrator, the normalized miR-210 expression of each tested sample was determined relative to the normalized miR-210 expression of the calibrator sample. Therefore, the amount of the miR-210 expression levels normalized to the expression of the *RNU44* and *RNU48* endogenous reference genes and relative to a calibrator is given by the $2^{-\Delta\Delta C_T}$ formula.

Statistical Analysis

Disease-free survival is defined as the time from resection of primary tumor to first recurrence or metastasis. Overall survival is defined as the time between primary resection of primary tumor to death for any reason. Because the distribution of miR-210 expression levels in patients was not Gaussian, *U*-tests were run to analyze the differential expression of miR-210 in relation to tumor status, namely primary and metastatic. The *U*-test was also conducted to evaluate the association of miR-210 expression levels, a continuous variable, with nominal parameters (eg, sex and laterality). In case of ordinal variables, such as tumor grade (I/II/III/IV), their relation with miR-210 expression levels (continuous variable) was estimated with the Jonckheere-Terpstra test. No cutoffs for miR-210 expression were established; hence, the X-Tile algorithm was used to produce an optimal cutoff of 2.75 relative quantification units (equal to the 57th percentile). According to this, patients were categorized into two groups (miR-210$^+$ and miR-210$^-$), and associations between miR-210 status and several clinicopathologic variables were examined with Fisher exact test or Pearson χ^2 test. The assessment of prognostic value of miR-210 for patients was performed with univariate and multivariate Cox proportional hazard regression and Kaplan-Meier analyses. The multivariate model was adjusted for patients' age, histologic stage, and tumor grade, and the *P* values were calculated by the test for trend approach. The Wilcoxon signed-rank test was used to compare the expression of miR-210 in the 40 pairs of ccRCC and adjacent normal kidney. Finally, the level of significance was defined as $P < 0.05$.

Clinical Validation with TCGA Data Set

We compiled miR-210 read counts and clinical variables associated with ccRCC patients from TCGA (*www.cancergenome.nih.gov*, last accessed December 8, 2013). Clinical variables that were analyzed in relation to miR-210 read counts included overall survival time (385 patients), pathologic stage (481 patients), and tumor size (423 samples). mRNA expressions of the predicted miR-210 targets was compared in 68 matched pairs of ccRCC tissues and adjacent normal kidney tissues, and read counts of miR-210 in 69 matched pairs of tissues were obtained from TCGA. In the statistical analysis of the TCGA data, the Kruskal-Wallis one-way analysis of variance test and the *U*-test were used to compare miR-210 expression with pathologic stage and tumor size, respectively.

Bioinformatics and Target Prediction Analysis

Target prediction was performed for miR-210 with TargetScanHuman 5.2 (*www.targetscan.org*, last accessed December 1, 2013) and miRecords (*www.mirecords.biolead.org*, last accessed December 1, 2013). The list of predicted gene targets was filtered by an extensive literature search and pathway analysis via DIANA-mirPath (*http://diana.cslab.ece.ntua.gr/pathways*, last accessed December 5, 2013) and the Gene Functional Classification tool from DAVID (Database for Annotation, Visualization, and Integrated Discovery) Bioinformatics Database (*http://david.abcc.ncifcrf.gov/gene2gene.jsp*, last accessed December 5, 2013).

Results

miR-210 Is Overexpressed in ccRCC Compared with Normal Kidney

We measured the expression of miR-210 in 40 pairs of normal kidney and ccRCC tissues from the same patient by using RT-qPCR analysis. miR-210 was significantly overexpressed in ccRCC tissues compared with their normal tissue counterparts ($P = 7.16 \times 10^{-15}$) (Table 1 and Supplemental Figure S1). These results were validated with data collected from TCGA, which showed significant upregulation of miR-210 in ccRCC tissues compared with adjacent normal kidney tissues ($P < 0.0001$) (Figure 1A). We compared miR-210 expression in unmatched 264 primary and 20 metastatic ccRCC samples. No statistically significant difference was found between miR-210 expression between primary and metastatic ccRCC (Supplemental Table S1).

Table 1 Pairwise Analysis of Clear Cell Renal Cancer Tissues and Adjacent Normal Kidney Tissues

Characteristic	Value
Total cases, *N*	40
Cases with pairwise* increase	38
Cases with pairwise decrease	2
Average miR-210 signal in normal tissues	64.88
Average miR-210 signal in cancer tissues	798.49
Pairwise fold change	16.25
Pairwise *P* value	7.16×10^{-15}

*Expression in tumor tissues compared with expression in adjacent normal tissues.

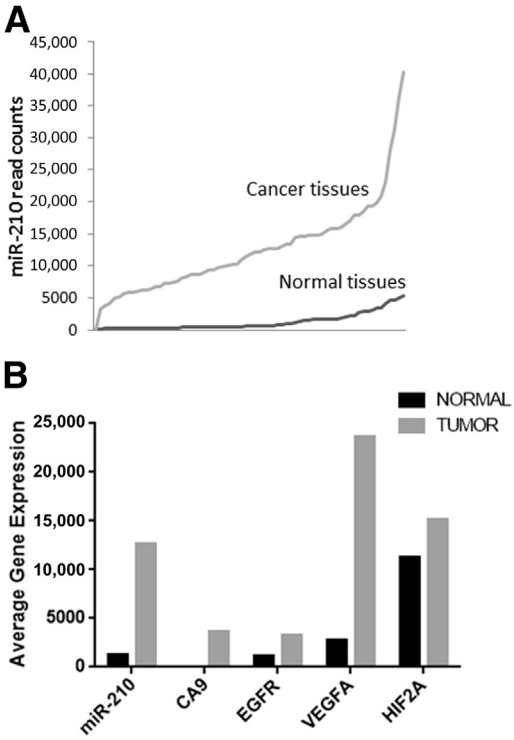

Figure 1 Validated miR-210 overexpression and hypoxia-related genes in ccRCC compared with normal counterpart from the same patient. **A:** miR-210 expressions in 69 pairs of normal kidney and adjacent ccRCC tissues were compiled from The Cancer Genome Atlas. Cases are shown on the *x* axis and expression levels on the *y* axis. **B:** Average normal and cancer expression values of miR-210 and hypoxia-related genes in the same cases. Similar to the RT-qPCR findings, miR-210 expression is higher in cancer tissues relative to normal tissues, and a similar trend is seen in the expression of hypoxia-related genes. ccRCC, clear cell renal cell carcinoma; RT-qPCR, quantitative real-time RT-PCR.

miR-210 Is a Prognostic Marker in ccRCC

We compared miR-210 expression in 264 samples of primary ccRCC. Distribution statistics are shown in Supplemental Table S1. As a continuous variable, no significant association was found between miR-210 expression and tumor size, stage, and grade ($P = 0.473$) (Supplemental Table S2). When used as a binary variable, miR-210 positivity was not associated with tumor size ($P = 0.455$), stage ($P = 0.564$), and tumor grade ($P = 0.186$) (Table 2).

miR-210$^+$ primary ccRCC patients had a statistically higher chance of disease recurrence or relapse [hazard ratio (HR), 1.82; 95% CI, 1.11−3.00; $P = 0.018$] (Table 3). In terms of overall survival, miR-210$^+$ patients also had significantly higher odds of shorter survival (HR, 2.46; 95% CI, 1.20−5.04; $P = 0.014$). Statistically significant associations with the use of the continuous miR-210 in disease-free survival and overall survival were not observed ($P > 0.05$).

After controlling for age and tumor grade and stage in the multivariate analysis, miR-210$^+$ tumors retained the statistically significant decrease of disease-free survival compared with miR-210$^-$ tumors (HR, 1.86; 95% CI, 1.07−3.24; $P = 0.028$). No significant results were obtained for overall survival (HR, 2.03; 95% CI, 0.88−4.62; $P = 0.094$). These results show that miR-210 is an independent prognostic marker compared with patient age, histologic stage, and tumor grade in ccRCC. When we adjusted the effect of tumor size and TNM stage in the multivariate Cox regression survival analyses of miR-210 with disease-free survival and overall survival, miR-210 was not found to be an independent prognostic marker, compared with these variables (data not shown).

Kaplan-Meier survival curves indicated that miR-210$^+$ patients had significantly lower disease-free survival ($P = 0.015$) and overall survival ($P = 0.011$) than did miR-210$^-$ patients (Figure 2). Further analyses were conducted for patient subgroups categorized on the basis of tumor size and stage. In the subgroup of patients with tumor size >4 cm, patients with miR-210$^+$ expression have statistically significant shorter overall survival ($P = 0.030$) (Figure 3). They also had shorter disease-free survival, although this did not reach statistical significance ($P = 0.077$). In the subgroup of patients with tumors ≤4 cm, miR-210$^+$ patients had lower disease-free and overall

Table 2 Associations between miR-210 Status and Clinicopathologic Variables of Cancer Patients

Variable	Total, *n*	Patients, *n* (%)		*P* value
		miR-210$^-$	miR-210$^+$	
Sex				
Male	171	93 (54.4)	78 (45.6)	0.238*
Female	91	57 (62.6)	34 (37.4)	
Age, years				
≤61	129	81 (62.8)	48 (37.2)	0.081*
>61	131	68 (51.9)	63 (48.1)	
Laterality				
Left	140	79 (56.4)	61 (43.6)	0.707*
Right	119	70 (58.8)	49 (41.2)	
Tumor size, cm				
≤5.5	133	78 (58.6)	55 (41.4)	0.455*
>5.5	126	68 (54.0)	58 (46.0)	
TNM stage				
I	93	55 (59.1)	38 (40.9)	0.564†
II	19	11 (57.9)	8 (42.1)	
III	28	19 (67.9)	9 (32.1)	
IV	34	17 (50.0)	17 (50.0)	
Histologic stage				
I	127	76 (59.8)	51 (40.2)	0.109†
II	40	16 (40.0)	24 (60.0)	
III	80	49 (61.2)	31 (38.8)	
IV	9	6 (66.7)	3 (33.3)	
Tumor grade				
I	14	11 (78.6)	3 (21.4)	0.186†
II	107	58 (54.2)	49 (45.8)	
III	99	58 (58.6)	41 (41.4)	
IV	35	16 (45.7)	19 (54.3)	

miR-210 status cutoff was 2.75 relative quantification units, equal to the 57th percentile.

*Calculated with Fisher exact test.

†Calculated with Pearson χ^2 test.

TNM, tumor, node, metastasis.

Table 3 miR-210 Expression and Patients' Survival

Variable	Disease-free survival		Overall survival	
	HR* (95% CI)	*P* value	HR* (95% CI)	*P* value
Univariate analysis				
miR-210				
Negative	1.00		1.00	
Positive	1.82 (1.11–3.00)	0.018	2.46 (1.20–5.04)	0.014
Age	1.01 (0.99–1.03)	0.34	0.99 (0.97–1.02)	0.84
Histologic stage (ordinal)	3.23 (2.00–5.21)	<0.001	3.47 (1.85–6.52)	<0.001
Tumor grade (ordinal)	3.58 (2.07–6.19)	<0.001	2.64 (1.37–5.08)	0.004
Multivariate analysis†				
miR-210				
Negative	1.00		1.00	
Positive	1.91 (1.01–3.31)	0.021	2.27 (1.01–5.12)	0.048
Age	1.01 (0.98–1.03)	0.56	0.99 (0.96–1.03)	0.69
Histologic stage (ordinal)	1.63 (1.19–2.22)	0.002	1.94 (1.20–3.12)	0.007
Tumor grade (ordinal)	2.55 (1.74–3.73)	<0.001	1.96 (1.17–3.28)	0.010

*Estimated from Cox proportional hazard regression model.
†Adjusted for patients' age, histologic stage, and tumor grade.
HR, hazard ratio.

survival, although this was not statistically significant (Supplemental Figure S2). Similar trends were found for the subgroup of patients with stage 1 disease (data not shown).

Validation of miR-210 Prognostic Significance

We validated our results in an independent data set of 481 cases of primary ccRCC from TCGA, which showed miR-210 expression to be independent from pathologic stage ($P = 0.5555$) and tumor size ($P = 0.5508$) (Figure 4, A and B). Kaplan-Meier curves showed that patients with higher miR-210 expression have statistically significant lower overall survival than patients with lower miR-210 expression ($P = 0.0257$) (Figure 4C). It should be noted, however, that a multivariate analysis is needed to confirm if miR-210 is an independent prognostic marker for disease-free and overall survival in the validation set. This was not performed because of lack of relevant clinical parameters in many cases of the validation set.

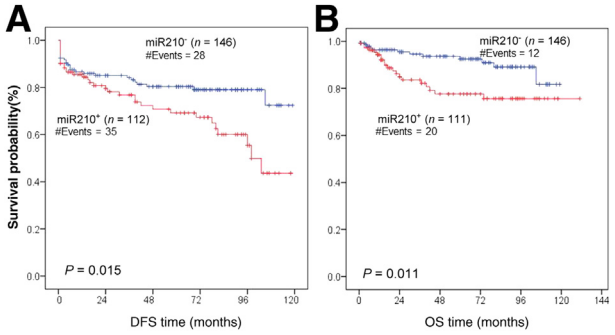

Figure 2 Kaplan-Meier curves for DFS and OS of patients with miR-210$^+$ and miR-210$^-$ tumors. miR-210$^+$ patients have significantly lower DFS (**A**) and lower OS (**B**) compared with miR-210$^-$ patients. DFS, disease-free survival; OS, overall survival.

Differential Expression of miR-210 in Different Subtypes of Kidney Cancer

The expression of miR-210 was compared in common subtypes of RCC, including clear cell, papillary, and chromophobe in addition to the benign oncocytoma. Average miR-210 expression was significantly higher in ccRCC than in normal kidney ($P < 0.0001$), although papillary carcinoma showed a comparable degree of up-regulation ($P < 0.0001$) (Figure 5). A much lower degree of up-regulation was found in chromophobe RCC ($P = 0.2443$), and levels of expression in oncocytoma were comparable with normal kidney.

Exploring the Role of miR-210 in ccRCC Pathogenesis

To gain more insight into the potential involvement of miR-210 in ccRCC pathogenesis and aggressive behavior, we performed target prediction analysis with multiple algorithms. We identified a number of predicted targets that can potentially be involved in carcinogenesis and the acquisition

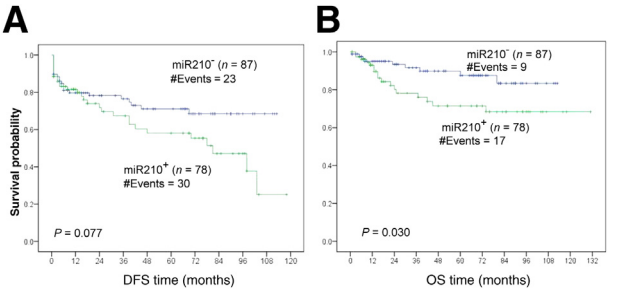

Figure 3 Kaplan-Meier curves for DFS and OS of patients within subgroup of tumors >4 cm. Within this subgroup, miR-210$^+$ patients had lower DFS (**A**) and lower OS (**B**) than miR-210$^-$ patients. DFS, disease-free survival; OS, overall survival.

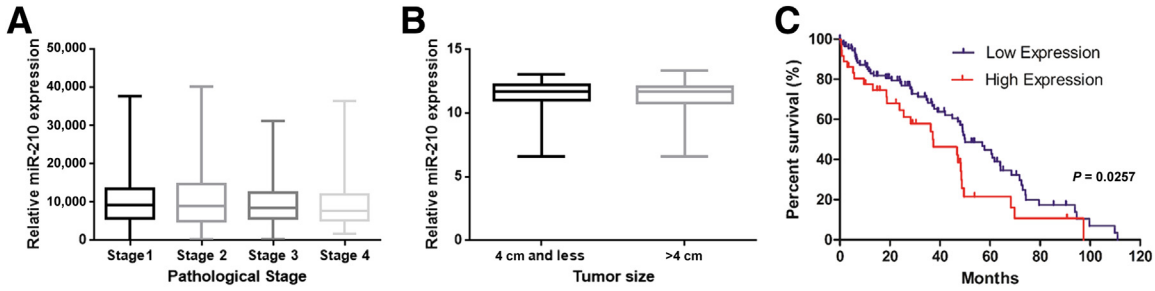

Figure 4 The correlation between miR-210 expression and tumor grade and stage. Cases are shown on the *x* axis and expression levels are on the *y* axis. miR-210 expression is not significantly associated with pathologic stage (**A**) and tumor size (**B**) in ccRCC patients. **C:** Kaplan-Meier survival curve shows that miR-210^{+} patents have statistically lower overall survival compared with miR-201^{-} patients ($P = 0.0257$). The horizontal line in the middle of each box indicates the median, and the top and bottom borders of the box mark the 75th and 25th percentiles, respectively. The whiskers above and below the box mark the 90th and 10th percentiles. ccRCC, clear cell renal cell carcinoma.

of aggressive behavior, based on published reports (Supplemental Table S3).[26–38] We also performed pathway analyses of the predicted targets, which revealed the involvement of the predicted gene targets in a number of carcinogenesis-related processes, including mitochondrial metabolism, stem cell survival, cell cycle regulation, angiogenesis, and cell-cell adhesion (data not shown).

To validate that miR-210 is hypoxia inducible, we examined the effect of hypoxia (induced by cobalt chloride) on miR-210 expression in the 786-O kidney cancer cell line. Our results showed that significant increase of miR-210 expression occurred on induction of hypoxia (data not shown). To further explore the relation between miR-210 and hypoxia, we examined the expression of a number of hypoxia-related genes, including vascular endothelial growth factor (*VEGFA*), *CA9, EGFR,* and HIF2A (*EPAS1*), and found a significant increase in their expression in cancer compared with normal cells. A positive correlation was found between the expression of these genes and miR-210 (Figure 1B). We further validated the correlation between miR-210 expression and vascular endothelial growth factor in an independent set of patient tumors with the use of PCR, and our results showed significant positive correlation between the two molecules (data not shown), indicating that miR-210 is hypoxia induced.

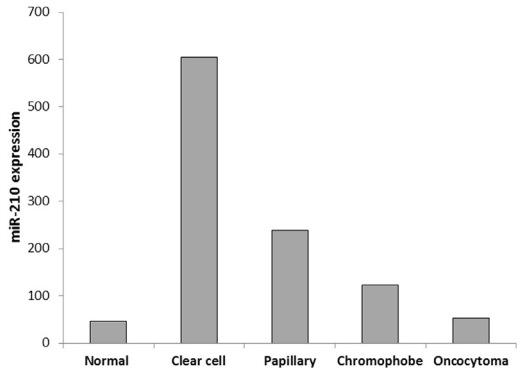

Figure 5 miR-210 expression in different subtypes of renal cell carcinoma and oncocytoma. miR-210 expression was quantified by RT-qPCR analysis. Average miR-210 expression is depicted for each subtype. miR-210 is most up-regulated in clear cell renal carcinoma compared with the other subtypes. RT-qPCR, quantitative real-time RT-PCR.

Discussion

Our results are consistent with recent literature that suggests the up-regulation of miR-210 in cancer and indicates that the hypoxia-mediated pathway is common to multiple malignancies. Similar to the up-regulation of miR-210 in ccRCC demonstrated in this study, miR-210 was also reported to be up-regulated in breast and pancreatic cancers, and its overexpression was connected to the hypoxic response in these malignancies.[19] Our findings are in agreement with a number of recent reports showing that miR-210 is a marker of poor prognosis in a number of cancers, including hepatocellular carcinoma, colorectal cancer, breast cancer, and osteosarcoma.[39–42] Among these reports is a meta-analysis[40] which showed that the expression of miR-210 in various carcinomas is related to poor prognosis. Moreover, reports show that miR-210 predicts poor survival in a number of cancers, including kidney cancer.[43] However, our results are in contrast with reports that show that miR-210 is associated with good prognosis in ccRCC.[20] Differences between the two studies can be attributed to technical variation or the use of different normalization techniques in addition to others. Factors related to intratumor and interpatient heterogeneity can also be responsible for variation in the results.

Our results show that miR-210 is an independent prognostic marker if the multivariate Cox regression model is adjusted to patient age, histologic stage, and tumor grade. TNM stage and tumor size are strong clinical variables, and it is difficult for one biochemical marker to significantly increase their prognostic significance.

One limitation of our study is that the continuous marker did not reach statistical significance, and the X-Tile dichotomization was a data-driven approach. Thus, the prognostic value and the cutoff for clinical utility still need to be investigated in future studies. Furthermore, because of data limitation, the validation was not done under a multivariate setting, so additional validation is still needed to confirm the

prognostic value of this marker. In addition, the multivariate analyses showed that this marker was associated with disease-free survival but not overall survival. Validation was only done for overall survival because of data limitation, meaning that further validation is still needed.

Our results point to the potential therapeutic utility of miR-210, whereby knocking down miR-210 could result in suppression of its oncogenic effect and result in arrest of tumor growth. This needs to be experimentally validated. Our data confirm previous research that shows the presence of a unique carcinogenic pathway for each of the RCC subtypes,[44] as indicated by the differential miRNA expression. Because miR-210 is a hypoxia-inducible molecule, it also emphasizes the activation of the von Hippel-Lindau/hypoxia pathway in ccRCC compared with the hematopoietic growth factor/met pathway in papillary RCC, the mammalian target of rapamycin pathway in chromophobe RCC, and the ubiquitin-proteasome system in oncocytoma.[45–47] In addition, recent reports have shown that miR-210 is a target of HIF-1 and HIF-2 and that there is an inverse correlation between von Hippel-Lindau expression and miR-210 expression levels.[20]

It was recently hypothesized that key molecules in RCC pathogenesis represent potential targets of miRNAs such as miR-210.[13,15,48] Several mechanisms by which miR-210 could be involved in the more aggressive behavior of RCC were suggested. One of the well-established mechanisms is the HIF-induced hypoxia response, whereby miR-210 is regulated by HIF-1 and HIF-2 leading to downstream effects.[19,20] Our data show that miR-210 has a number of other targets that contribute to ccRCC pathogenesis through a number of distinct mechanisms (Supplemental Table S3). However, some of these effects might be indirect because of the involvement of intermediate molecules, and the function of each miRNA can be specific for cell type and tissue. Interestingly, recent evidence also suggests that miR-210 can regulate HIF-1, indicating interplay between miR-210 and the hypoxic response.

Recent studies suggest the presence of distinct biological subtypes of ccRCC on the basis of HIF-α expression pattern and whether von Hippel-Lindau–deficient ccRCC tumors express both HIF-1α and HIF-2α or only HIF-2α.[5,49] Downstream effectors, such as miR-210, could explain the aggressive behavior of some of these subtypes.

Our results show that miR-210 expression is an independent prognostic factor with no correlation with clinical parameters such as tumor stage or size. Integrating molecular markers with clinical parameters could enhance the ability to divide the patient population into smaller well-defined groups with specific clinical behavior and could allow better selection of treatment to fit individual patient needs.[50–52] Many potential prognostic and predictive molecular biomarkers are now identified in RCC, although none has yet entered into clinical practice, and all require prospective validation in appropriately designed randomized studies. In the near future, however, validated biomarkers may become integral to management strategies in RCC, enabling tailored treatment for individual patients to improve clinical outcomes.[53]

Further investigations will establish the role of miRNAs as noninvasive multipurpose serum and/or urine biomarkers for kidney cancer. Recent data confirm that miRNAs are actively secreted and are readily measurable in body fluids. Another great advantage of miRNAs as tumor markers is that they are stable, even in formalin-fixed, paraffin-embedded tissues. They also can be measured in formalin-fixed tissues by *in situ* hybridization.

In addition to their potential prognostic significance as demonstrated in this study, miRNAs could serve as indicators of complete removal of tumor after surgery. Although a single miRNA might not have the desired sensitivity and specificity to provide an accurate assessment of prognosis, the use of a combination of these miRNAs, which can be easily measured in a single multiparametric test, could enhance the performance of these biomarkers.

The choice of therapy for each individual patient with metastatic RCC is still empirical, and most available therapies rely on targeting the vascular endothelial growth factor and mammalian target of rapamycin pathways. A more thorough understanding of the molecular basis of pathogenesis that may be unique in each patient could help direct treatment decisions. Moreover, miRNAs represent potentially attractive therapeutic targets that could potentially be used as adjuvant therapies to prevent development of resistance to targeted therapy.

Conclusion

We have shown that miR-210 is significantly overexpressed in ccRCC with higher expression in metastatic tumors. Higher miR-210 expression can serve as an independent marker of poor prognosis in ccRCC. Higher levels of miR-210 are seen in the clear cell and papillary subtypes, compared with chromophobe RCC and oncocytoma. We identified a number of predicted targets that could potentially be involved in carcinogenesis and the acquisition of aggressive behavior. These results suggest an active involvement of miR-210 in RCC pathogenesis and its potential utility as a therapeutic target.

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authors contributed to study design, data interpretation, and drafting of the manuscript.

Supplemental Data

Supplemental material for this article can be found at *http://dx.doi.org/10.1016/j.jmoldx.2014.10.005*.

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