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UTILITY OF DIFFERENT MICRORNA AND IMMUNOHISTOCHEMICAL PANEL TO DIFFERENTIATE RENAL CELLCARCINOMA SUBTYPES AND ITS CORRELATION TO THE DISEASE OUTCOMES.

Mohamed H Zahran[1*], Amira Awadalla[2], Asmaa E Ahmed[3], Ahmed E Elbatta[4], Shery Khater[5],Essam Elsawy[6], Hassan Abol‐ Enein[7], Ahmed A Shokeir[8], Ahmed Mosbah[9], Ahmed S. El- Hefnawy[10*]

[1*,4,7,8,9,10*]Urology department, Urology and Nephrology Center, Mansoura University, Egypt
[2,3]Center of Excellence for genome and cancer research, Urology and Nephrology Center, Mansoura University, Egypt
[5]Pathology department, Urology and Nephrology Center, Mansoura University, Egypt
[6]Microbiology department, Urology and Nephrology Center, Mansoura University, Egypt

Corresponding author: Mohamed H Zahran
Associate professor of urology, Urology and nephrology center, Mansoura University, Egypt
Email: zahranmha822@gmail.com, zahranmha@mans.edu.eg
ORCID ID: 0000-0002-2897-1023

Corresponding author: Ahmed S. El Hefnawy
Professor of urology, Urology and nephrology center, Mansoura University, Egypt
Email**:** a_s_elhefnawy@yahoo.com
ORCID ID: 0000-0001-9357-501X

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ABSTRACT:

Objectives: To differentiate between renal cell carcinoma (RCC) subtypes through the expression of different microRNAs and immunohistochemical markers and its correlation to the disease outcomes.
Methods: We examined the stored fresh frozen specimen of 137 RCC and adjacent healthy renal tissue for the quantitative RT-PCR expression of five microRNAs (miRNA222, miRNA221, miRNA126, miRNA200b, miRNA200c) and the immunohistochemical staining severity of AMACR, CK7, CD10 and CD11. The relative expression of the markers were compared between different RCC subtypes and correlated to the disease stage, grade and recurrence.
Results: Clear RCC (cRCC) can be differentiated from other subtypes by the higher expression of miRNA126 ($P<0.001$, ≥ 7.5 has 98% sensitivity and 95% specificity) and staining for CD10 ($P<0.001$). However, miRNA126 did not correlate with the stage ($r2=0.1$, $p= 0.2$), grade ($r2=0.04$, $p=0.07$), L.N stage ($r2=0.05$, $P=0.6$) or disease recurrence ($r2=0.06$, $p=0.5$). Papillary RCC (pRCC) can be differentiated from chRCC and oncocytoma by higher expression of miRNA 221 and higher staining for AMCAR and CD10 and less staining for CD117 ($p<0.001$). Chromophobe RCC (chRCC) showed significantly higher expression of miRNA 200c. MiRNA 200c expression ≥ 0.43 has 70% sensitivity and 70% specificity for detection of chRCC (AUC=0.7 and P= 0.001).
Conclusion: Different microRNAs together with the immunohistochemical markers seem to be a useful clinical tool to differentiate RCC subtypes. Further studies are required to study its implication on the disease management outcome and response to new targeted therapy.

Keywords: Renal cell carcinoma, nephrectomy, genetic testing, miRNA, immunohistochemical panel

1. Introduction

Renal cell carcinoma (RCC) is the third most common urogenital malignancy and accounts for 2-3% of all cancers in adults. The incidence of RCC increases worldwide over the last decades. Wide use of radiological investigations increased detection of early and asymptomatic cases. However, 20-30 % have metastasis at time of presentation and one third may develop metastasis after surgical treatment [(1)]. RCC encompasses different histologic subtypes with distinct genetic and molecular characteristics. Clear cell RCC (cRCC), papillary RCC (pRCC) and chromophobe RCC (chRCC) represent > 90% of the diagnosed cases.

Unclassified subtype represents 4–5% of RCC cases. The benign form, oncocytoma; representsup to 25% of excised tumors at early stage [2]. RCC classification depends mainly on histopathological and immunohistochemistry examination. This is not always accurate, especially in small biopsies; because of the inter-observer variability and the overlapping morphological features of different sub-types. Accurate diagnosis is of utmost importance because of the different clinical and prognostic behavior of the different subtypes and the different response to therapeutic approaches.

RCC subtypes exhibit different chromosomal abnormalities which would be reflected on the expression of different miRNAs; which play a pivotal role in cell proliferation, differentiation, invasiveness, apoptosis, hormone secretion, angiogenesis and cell cycle control. MiRNAs are highly specific, stable and can be easily extracted from body fluids and tissues.

Different studies have been demonstrated the expression of different miRNAs in RCC and correlated its expression to the RCC subtypes. Some correlated its expression to the disease stage, metastasis and prognosis [3-7]. So, it seemed to be a promising diagnostic, prognostic andpredictive biomarkers for RCC [8].

Different immunohistochemical markers have been identified with different level of expression in different subtypes of RCC. Alpha-methyl CoA racemase (AMACR) and CK7 were highly expressed in pRCC but with limited prognostic impacts [9, 10]. CK7 could differentiate chRCC from oncocytoma where CD117 was highly expressed in in chRCC and oncocytoma but not in cRCC [11]. CD10 was expressed extensively in cRCC and associated with low stage, grade and better prognosis. Also, it was highly expressed in in pRCC type 2 than type 1. On the other hand, it was less expressed in chRCC [12].

Both miRNAs and different immunohistochemical markers could be used to accurately identify different RCC subtypes and overcome the drawbacks of classical histopathological examination [2]. We assessed the quantitative RT-PCR expression of five different miRNAs and immunohistochemical panel of four markers in RCC and compared its expression and its accuracy (sensitivity and specificity) in differentiation between different RCC subtypes and correlated the miRNA expression to the disease stage, grade and recurrence. The manuscript wasprepared in accordance with the STARD-2015 reporting checklist [13].

2. Methods

After obtaining institutional review board approval (RP/19.07.37), we conducted a retrospective analysis of 137 stored RCC specimens at the center of excellence for genome and cancer research, Mansoura Urology and Nephrology Center. The specimens were assessed for the expression of five miRNA and four immunohistochemical markers. The stored specimens were collected from malignant and adjacent normal renal tissue after radical nephrectomy and nephron sparing surgery for RCC between January 2014 and December 2019. The specimens were stored at -80 ◦C. The specimen were reviewed according to the latest ISUP classification of RCC [14]. All demographic data of the patients were retrieved from the dedicated electronic database including age, sex, BMI, preoperative laboratory results, disease stage, grade, subtypes, operative details and follow-up data.

MiRNA gene expression

Formalin-fixed paraffin-embedded sections were used for RNA extraction. Five cores from the pure tumour tissue were selected to compensate for heterogeneity and five cores from adjacent normal tissue were collected for examination. Tissue was extracted from areas without evident hemorrhage or necrosis. MiRNA was extracted from tissue samples using

miRNeasy mini kit (Qiagen, Hilden, Germany). MiRNA was reverse transcribed to cDNA using miScript Reverse Transcription kit (Qiagen, Hilden, Germany). MiScript SYBR-Green PCR kit (Qiagen, Hilden, Germany) and miScript primers (miRNA-221, miRNA-222, miRNA-126, miRNA-200band miRNA-200c) were used for qPCR assays and normalized to RUN6-2 (Qiagen, Hilden, Germany). Data analysis was performed using the following equation $2^{-\Delta\Delta CT.}$

Immunohistochemical examination

It was carried on three µm-thicknesses sections. The hydrogen peroxide was used in the treatment the deparaffinized sections then heated in citrate buffer. Then it was incubated at room temperature with the monoclonal antibodies of AMACR, CK7, CD10 and CD117 (monoclonal mouse anti-human antibody, DAKO, USA). Immunostaining was achieved using Power-Stain™

1.0 Poly HRP AEC Kit (Genemed Biotechnologies, San Francisco, CA, USA) with (DAB) as a chromogen. Olympus CX51 light microscope was used to examine the slides. The positivity was identified by cytoplasmic staining for AMACR, cytoplasmic staining with membranous accentuation for CK7, and membranous staining for CD10 and CD117.

The score of these markers was calculated through multiplying the staining intensity (1= weal, 2= moderate and 3= marked) by the percentage of positive cells: 0= negative staining in alltumour cells, 1= > 10 % of tumour cells shows mild positive staining, 2= 20-30 % of cells showsmoderate positivity and 3= strong positive staining of more than 60% of cells.

Outcome:

The primary outcome was to compare the expression of different miRNAs between RCC and normal renal tissue. Second, we tested the differences in expression of different miRNA and immunohistochemical markers between different RCC subtypes. The cut-off value of miRNA associated with higher sensitivity and specificity for detection of RCC subtypes was determined. The expression of miRNA and immunohistochemical markers expression were correlated to the disease stage, grade and recurrence.

Statistical analysis:

Continuous data including miRNA quantity was described as median and IQR and categorical variables were expressed as number and percentage. Comparison of median values was done by Mann-Whitney U test, where categorical value was compared using Chi-square test. Correlationof the miRNA expression and disease stage, grade and lymph node stage was done using Spearman correlation coefficient. Receiver operating characteristics (ROC) curve was used to identify specify the miRNA cut-off values for detection of RCC subtypes. All statistical tests were carried out using IBM ‘’SPSS’’ statistics version 21, with a P value of less than 0.05 was considered significant.

3. Results

Clinical data:

The study included 71, 12, 36 and 18 cRCC, pRCC type I, chRCC and oncocytoma, respectively.The clinical data and demographics of the included cases are depicted in **table1**.

MiRNA expression in RCC and normal renal tissue

RCC specimen showed statistically significant higher expression of miRNA-221, miRNA-222, miRNA-126 ($P<0.001$) and lesser expression miRNA-200b and miRNA-200c($P<0.001$) than normal renal tissues. (**Figure1)**

MiRNA expression indifferent RCC subtypes:
Clear RCC has significantly higher expression of miRNA126 compared to other subtypesof RCC. Higher expression of miRNA 126 (≥ 7.5) has 98% sensitivity and 95% specificity for detection of cRCC (AUC= 0.96, P<0.001). However, it did not correlate with the disease stage (r^2=0.1, p= 0.2), grade (r^2=0.04, p=0.07), L.N stage (r^2=0.05, P=0.6) and disease recurrence (r^2=0.06, p=0.5). Clear RCC can be differentiated from pRCC by the significant higher expression of miRNA126 and miRNA222. Clear RCC and pRCC can be differentiated from chRCC and oncocytoma by the significant lower expression of miRNA221. However, miRNA221 significantly correlate with disease recurrence in cRCC (r^2=0.4, p= 0.01) but not significantly correlate with disease stage (r^2=0.02, p= 0.2), grade (r^2=0.05, p= 0.5) or LN stage(r^2=0.03, p= 0.6).
Chromophobe RCC can be differentiated from oncocytoma by the significant higher expression of miRNA222, 200b and 200c. Higher expression of miRNA 200c (≥0.43) has 70% sensitivity and 70% specificity for detection of chRCC (AUC=0.7 and P= 0.001) **(Table2)**

Immunohistochemistry staining of RCC
For cRCC, all specimens were stained positive for CD10 which differentiate it from other subtypes. However, It did not correlate with stage (r^2=0.03, p= 0.1), grade (r^2=0.02, p=0.2), L.N stage (r^2=0.09, P=0.8) or disease recurrence (r^2=0.1, P=0.2). 90% stained positive for AMACR but most were mildly stained (79%). It did not correlate with stage (r^2=0.01, p= 0.4), grade (r^2=0.01, p=0.3), L.N stage (r^2=0.04, P=0.1) or disease recurrence (r^2=0.25, P=0.2). **(Figure 2)** Only 11% and 1 % stained mildly for CK7 and CD117, respectively.
For pRCC, 92 % and 83% stained positive for AMACR and CK7 respectively. On the other hand 34% and 8% stained mildly for CD10 and CD117, respectively. Chromophobe RCC can be differentiated from both clear and pRCC by absence of staining for AMACR and CD10. Compared to cRCC, chRCC showed higher staining for CK7 and CD117 (P<0001) and it can bedifferentiated from pRCC by the higher staining for CD117 (P<0.001). **(Figure 3)**
Oncocytoma can be differentiated from the clear and pRCC by absence of staining for CD10 (P=<0.001 and 0.009, respectively) and higher grades of staining for CD117 (P<0.001). Few specimens (17%) stained mildly for AMCAR unlike chRCC which did not stain for it. Despite, all specimens were stained positive for CD117 as chRCC, the severity of staining is significantlyless than chRCC (p<0.001). **(Table 3)**

4. Discussion

Despite the advances in the therapeutic approaches, the prognosis of RCC remains poor especially for advanced cases with 5-year survival of 13 % [(15)]. Proper understanding of the tumour microenvironment is crucial for better understanding the heteterogenicty of different subtypes and for development of new targeted therapy in this era of immuno-oncologic agent-based therapies [(14)].
We developed a miRNA and immunohistochemical panel- based assay that can help in differentiation between RCC subtypes and oncocytoma. We found that miR-221 could distinguish cRCC and pRCC from chRCC and oncocytoma. Similarly, significant elevation of miR-221 levels in chRCC and oncocytoma relative to cRCC and pRCC has been reported [(2)]. MiRNA 221 is significantly up-regulated both in tissues and circulation of RCC patients compared to normal healthy individuals[(6)]. Moreover, higher circulating level was identified in patients with metastatic RCC than those with no metastasis [(6)]. MiR-221 promotes cell proliferation, mobility, and inhibits cell apoptosis in cell lines. High expression of miR-221 hadbeen associated with poor prognosis and shorter overall survival[(5, 6)]. Similarly, we found

that higher miRNA221 was correlated with disease recurrence in patient with cRCC.

MiRNA 126 has high sensitivity and specificity for diagnosis of cRCC. Higher expression of miR-126 was noted in cRCC compared to pRCC[2-4]. miRNA126 is a marker ofangiogenesis which is a prominent feature of cRCC [16]. Down-regulation of miRNA126 was associated with high stage and high grade of cRCC. Also, low expression of miRNA126 was associated with shorter time to recurrence and associated with statistically significant lower patient survival [3, 4]. However, we could not identify a correlation between its level and diseasestage, grade or recurrence.

Both miRNA221 and 222 play a critical oncogenesis role in many tumours including RCC. They modulate cell cycle by suppressing cell cycle inhibitory proteins and facilitating cell proliferation [17]. Also, they influence phosphatase and tensin homolog (PTEN) tumour suppressor gene expression which up-regulated Akt, resulting in cellular proliferation, invasion and inhibition of apoptosis. Inactivation of PTEN and up-regulation of Akt was associated with radio-resistance and resistance to other anti-neoplastic therapies. This effect could be reversed bymiRNA 221 and 222 knockdown which resulted in restoration of PTEN level and enhancement of radiation induced-apoptosis [18]. Both miRNAs were highly expressed in the RCC tissue than in healthy tissue as shown her in the study results. Also, they are reported to increase in the serum of RCC patients. Higher level was associated with poor prognosis and lower overall survival [6, 19]. Herein, miRNA 222 was not correlated with cRCC stage, grade or disease recurrence.

Similar to previous reports [7, 20], we found that miRNA-200 b and c were significantly less expressed in RCC compared to normal renal parenchyma. They function as a RCC suppressor as restoration of miR-200 family resulted in significant inhibition of RCC cell proliferation and migration[20]. We could differentiate chRCC and oncocytoma using MiR-200 family with miR-222 expression. Similarly, higher expression of miR-200b and miR-200c was reported in chRCC compared to oncocytoma. In addition, higher miRNA200c was proved to be specific to chRCC [2]. We suggested a practical immunohistochemical panel of AMACR, CK7, CD10 and CD117 to cover almost all RCC subtypes. AMACR is a mitochondrial enzyme expressed normally in hepatocytes and proximal renal tubular and bronchial epithelium. It mediates the process of fatty acid oxidation. It is a sensitive marker for different genitourinary malignancies. Diffuse and strong cytoplasmic staining is a characteristic of pRCC and its deficiency is a markerof tumors derived from distal nephrons such as oncocytoma and chRCC [21] and expressed rarely in cRCC[22]. Similarly, we found that cRCC was largely negative or with focal mild cytoplasmic staining for this marker, where pRCC was largely positive for it.

Most cRCC and oncocytoma were negative for CK7, whereas chRCC showed moderate positivity in nearly 56% of the cases. Our results coincided with El-Shorbagy and his colleagues who found that most cRCC and oncocytoma were negative for CK7 (91.7% and 83.3%, respectively), compared to chRCC, which showed positivity in 86% of the cases [23]. Another study showed that CK7 was expressed at low levels in cRCC; negative or occasionally focal positive, as well as in pRCC which often showed patch-positive insufficient for positive interpretation. Whereas diffuse CK7 positivity is an indicator of chRCC (100%), [22].

The present study showed CD117 negative expression for cRCC and pRCC and markedly positive in nearly 50% of oncocytoma and 94% chRCC. Such findings coincided with those of Liu et al who reported that CD117 was strongly expressed in 82% of chRCC and all oncocytoma, whereas cRCC was not immunoreactive. CD117 had 100% specificity and 90% sensitivity in differentiation of cRCC from both chRCC and oncocytoma [24]. Also, Pan et al found that 83% of chRCC and 71% of oncocytoma had membranous immunoreactivity for CD117, whereas all cRCC was negative [25] .

CD10 has been documented as a poor prognostic factor in genitourinary tract cancer. It can differentiate cRCC from pRCC, according to the pattern of staining [22]. Herein, cRCC showed diffuse membranous staining for CD10 unlike pRCC which showed no or focal staining. None of the other subtype showed immunoreactivity for CD10. Previous studies reported CD10 immunoreactivity in cRCC and pRCC and absence of reactivity in chRCC [26, 27]. On the other hand, others identified immunoreactivity of chRCC for CD10 and its expression was associated with disease aggressiveness [28].

This study has some limitations. Being a retrospective with inherent selection bias and limited to a small patients' numbers were the main limitation. These could affect the results of the markers expression correlation to the disease criteria and outcomes. This can be explained by the limited available stored specimen. It included the commonest subtypes of RCC and missing some subtypes like papillary type 2. Prospective multicenter studies are required to validate the reliability and reproducibility of the results. Further studies are required to identify the correlation between miRNA expression and disease outcome and its relevance to the newly available targeted therapy in advanced diseases.

In conclusion, using the miRNA and immunohistochemical panel, we can differentiate cRCC from other subtypes by the higher expression of miRNA126 and immune-reactivity for CD10. Chromophobe RCC can be differentiated from oncocytoma by higher miRNA200 b and c expression and diffuse CK7 immunoreactivity.

Authors' contribution:

Authors	Contributions
Mohamed H Zahran	Conceptualization, data curation, formal analysis, methodology, writing , review ad editing
Amira Awadalla	data curation, methodology, resources, project administration, validation, supervision
Asmaa E Ahmed	Data curation , investigation
Ahmed E Elbatta	Data curation
Shery Khater	Investigation, methodology
Essam Elsawy	Investigation, supervision
Hassan Abol‑ Enein	Methodology, supervision, writing-review and editing
Ahmed A Shokeir	Conceptualization, Methodology, supervision, writing-review and editing
Ahmed Mosbah	Methodology, supervision, writing-review and editing

Ahmed S. El Hefnawy	Conceptualization, funding acquisition, methodology, project administration, supervision, writing-review and editing

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Conflict of interest: Authors have no conflict of interest to disclose.

Ethics statement:

The study was approved by *Mansoura* Faculty of Medicine Institutional Research Board (MFM- *IRB*) as a part of a project entitled (Hepatitis C virus and renal cell carcinoma. Is there a possible correlation) and the approval number is approval (RP/19.07.37)

As the study is a retrospective one and included mainly stored histopathological specimen, theInformed consent was exempted by the MFM-IRB committee for this purpose.

Informed consent: N/A

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Legend to figures:

Figure 1: Different miRNA expression in RCC and normal kidney tissues (All p values <0.001).

Figure 2: Staining of Renal cell carcinoma (RCC) for CD10; Diffuse (90%), moderately intense (+2) membranous staining in Clear RCC (A), negative staining in papillary RCC (B), negative staining in Oncoytoma (C), and Chromophobe RCC (D). And staining of RCC for AMACR showing Focal (10%), mildly intense (+1) cytoplasmic staining in Clear RCC (E), Diffuse (100%), moderately intense (+2) cytoplasmic staining in papillary RCC (F), negative staining in Oncoytoma (G), and Chromophobe RCC (H), x:100.

Figure 3: Staining of Renal cell carcinoma (RCC) for CK7 showing negative staining in Clear RCC (A), Diffuse (100%), markedly intense (+3) cytoplasmic staining in papillary RCC (B), Diffuse, positive, cytoplasmic staining of mild (+1) intensity in Oncoytoma (C), and Diffuse, positive, membranous and cytoplasmic staining of moderate (+2) intensity in Chromophobe RCC (D). And staining of RCC for CD117 showing negative staining in Clear RCC (E), negative staining in papillary RCC (F), Diffuse, positive, membranous staining of marked (+3) intensity in Oncoytoma (G), and Diffuse, positive, membranous staining of marked (+3) intensityin Chromophobe RCC (H), x:100.

Table 1: Demographic and clinical characteristics of the included renal cell carcinoma patients.

Characteristics	**Number (%)**
Age. *years Median (IQR)*	58(49-64)
BMI *Median (IQR)*	30(26-34)
Sex: *Male Female*	94 (68.6%) 43 (31.4%)
Symptoms :*Incidental Pain Hematuria*	61 (44.5%) 53 (38.7%) 23 (16.8%)
Medical Co-morbidites: *HypertensionDM* *Both*	38 (27.7%) 15(10.9%) 21(15.3%)

Laboratory results: *Median (IQR)* **Cr**.*mg/dl* **HB**. *gm/dl* **Albumin.** *gm/dl* **Platelets.** *X10^3*	1 (0.8-1.3) 12.1(11.2-13.5) 4.1(3.8-4.3) 209 (170-276)
Tumor size: *greatest dimensionMedian (IQR). cm*	8.6 (6.8-10.5)
pT *T1aT1bT2aT2bT3 T4*	4 (2.9%) 22 (16.1%) 22 (16.1%) 17 (22.4%) 61 (44.5%) 11 (8%)
pN *N0N1*	122 (89.1%) 15 (10.9%)
Recurrence	5 (3.6%)
Metastasis	13 (9.5%)
Follow-up. *MonthsMedian (IQR)*	19.5 (6-40.7)

Table 2: miRNA expression indifferent subtypes of renal cell carcinoma.

Median (IQR)	**cRCC**	**pRCC**	**chRCC**	**Oncocytoma**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
miRNA222	3.5 (2.6-3.9)	1.8 (1.5-3.7)	3.9 (3.8-4.2)	1.8 (1.6-2.8)	0.002	0.001	<0.001	0.004	0.9	<0.001
miRNA221	3.2 (2.9-3.6)	2.8 (2.4-3.4)	4.9 (4.7-7.9)	**5.5 (5-6.6)**	0.9	<0.001	<0.001	<0.001	<0.001	0.1
miRNA126	**14.3 (13.9-15.2)**	1.5 (1.3-11.2)	1.7 (1.4-1.9)	1.7 (1.4-1.9)	<0.001	<0.001	<0.001	0.9	0.5	0.8
miRNA200b	0.33 (0.3-0.5)	0.4 (0.23-0.6)	**0.5 (0.330.7)**	0.16 (0.12-0.32)	0.8	0.03	<0.001	0.3	0.01	<0.001
miRNA200c	0.4 (0.32-0.42)	0.38 (0.26-0.46)	**0.5 (0.35-0.54)**	0.12 (0.08-0.25)	0.7	0.04	<0.001	0.1	0.01	<0.001

P1: cRCC vs. pRCC, **P2**:cRCC vs.chRCC, **P3**: cRCC vs. oncocytoma, **P4**:pRCC vs. chRCC , **P5**: pRCC vs. Oncocytoma, **P6**: chRCC vs. oncocytoma.cRCC: clear Renal cell carcinoma, pRCC: papillary RCC, chRCC: chromophobe RCC

Table 3: Immunohistochemical staining of different renal cell carcinoma subtypes.

	ccRCC	**pRCC**	**chRCC**	**Oncocytoma**	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**
AMACR					0.8	<0.001	<0.001	<0.001	<0.001	0.01
Negative	7 (10%)	1 (8%)	36 (100%)	15 (83%)						
Mild	56 (79%)	3(25%)	0	3(17%)						
Moderate	5 (7%)	6 (50%)	0							
Marked	3 (4%)	2(17%)	0							
CK7					<0.001	<0.001	<0.001	0.4	0.4	0.05
Negative	63 (89%)	2 (17%)	3 (8%)	5(28%)						
Mild	8 (11%)	2 (17%)	13 (36%)	13 (72%)						
Moderate	0	2 (17%)	20 (56%)	0						
Marked	0	6 (50%)	0	0						
CD10					<0.001	<0.001	<0.001	0.003	0.009	0.4
Negative	**0**	8 (67%)	35 (100%)	18 (100%)						
Mild	**5 (7%)**	2 (17%)	0	0						
Moderate	**33 (46.5%)**	0	0	0						
Marked	**33 (46.5%)**	2 (17%)	0	0						
CD117					0.1	<0.001	<0.001	<0.001	<0.001	0.9
Negative	70(99%)	11(92%)	0	0						
Mild	1 (1%)	1 (8%)	0	0						
Moderate	0		**2(6%)**	**9 (50%)**						
Marked	0		**34 (94%)**	**9 (50%)**						

P1: cRCC vs. pRCC, **P2**:cRCC vs.chRCC, **P3**: cRCC vs. oncocytoma, **P4**:pRCC vs. chRCC , **P5**: pRCC vs. Oncocytoma, **P6**: chRCC vs.oncocytoma. cRCC: clear Renal cell carcinoma, pRCC: papillary RCC, chRCC: chromophobe RCC

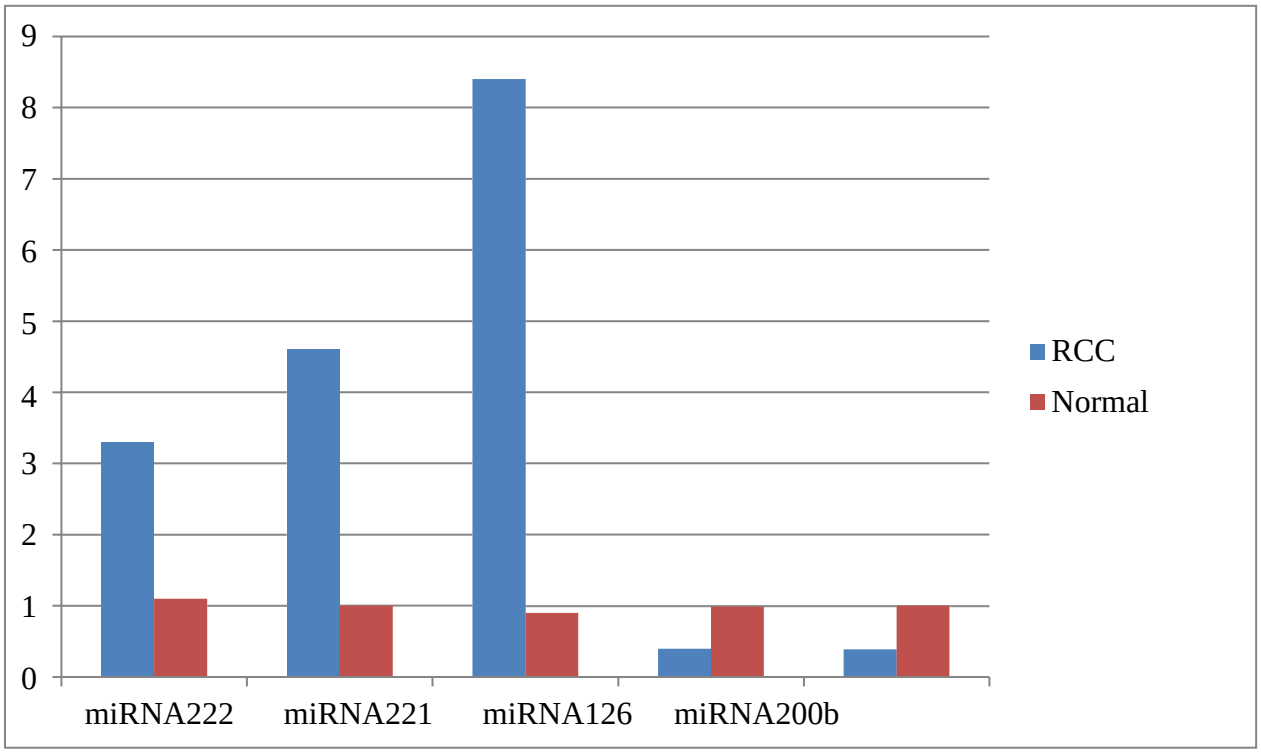

Figure 1

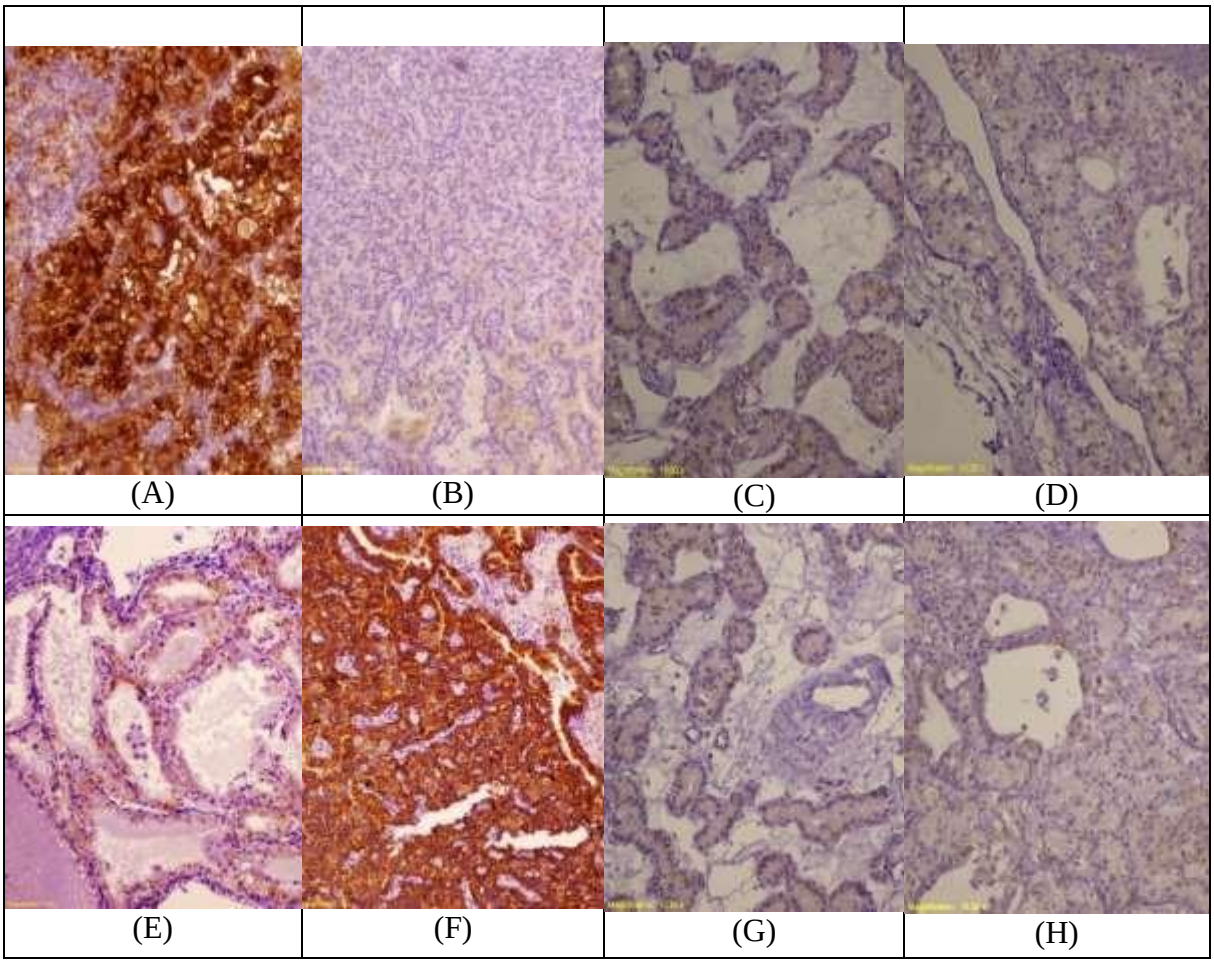

Figure 2

Figure 3