in vivo *31*: 625-630 (2017)
doi:10.21873/invivo.11103

MicroRNA-1 and MicroRNA-21 Individually Regulate Cellular Growth of Non-malignant and Malignant Renal Cells

HANNES AHREND[1*], ANNE KAUL[2*], SUSANNE ZIEGLER[3], LARS-OVE BRANDENBURG[4], UWE ZIMMERMANN[1], ALEXANDER MUSTEA[2], MARTIN BURCHARDT[1], PATRICK ZIEGLER[3] and MATTHIAS B. STOPE[1]

Departments of [1]Urology and [2]Gynaecology and Obstetrics, University of Medicine Greifswald, Greifswald, Germany; [3]Institute for Occupational and Social Medicine and [4]Department of Anatomy and Cell Biology, RWTH Aachen University, Aachen, Germany

Abstract. *Background/Aim: Due to its poor prognosis, it is increasingly necessary to understand the biology of renal cell cancer (RCC). Therefore, we investigated the role of microRNAs miR-1 and miR-21 in the growth of RCC cells compared to that of non-malignant renal cells. Materials and Methods: Four malignant cell lines (Caki-1, 786-O, RCC4, A498) were examined regarding their cell growth, microRNA and telomerase expression, and were compared to non-malignant RC-124 renal cells. Results: Inconsistencies appeared in the panel of RCC cells regarding antiproliferative and proliferative properties of miR-1 and miR-21, respectively. Notably, and most likely due to immortaliziation, non-malignant RC-124 cells exhibited telomerase expression and activity. Conclusion: miR-1 and miR-21 functionality in cancer progression, particularly in tumor growth, may be more dependent on the individual cellular context and may reflect RCC heterogeneity. Thus, both microRNAs, in combination with other stratifying biomarkers, may be useful in terms of RCC diagnosis, prognosis, or treatment response.*

Tumorigenesis gives cells an advantage in survival by an increase of proliferation, angiogenesis, immunomodulation and immortality (1-4). Due to its delayed diagnosis until advanced stages, renal cell cancer (RCC) is known as the deadliest neoplasm of the urinary tract (1, 5). Thus, it is more necessary than ever to understand its tumor biology and explore new targets for further therapeutic approaches and prognostic options (6). Former studies suggested that among others, a misguided microRNA expression pattern may be involved in tumor initiation and progression in RCC (7, 8). Among such mechanisms, small regulatory RNAs, so-called microRNAs (miRs), can operate as controllers of different proccesses in tumorigenesis, such as cell migration and differentiation, as well as metastasis and apoptosis (9-11). *miR-1* and *miR-21* are two of numerous representatives of microRNAs. *miR-1*, initially found in cardiac muscle cells (12), but also found in various tissues (13-15), was strongly suspected of acting as a tumor suppressor due to its anti-oncogenic properties (16) in prostate, breast and colorectal cancer (13-15); interestingly, the type of tissue in fact determines the biological behavior of *miR-1* (9). Sadly, the current data situation for the expression of *miR-1* in RCC leaves much to be desired. For this reason, our study aimed to expand the first findings in order to gain a better understanding of the tumor biology of RCC. Similarly to *miR-1*, *miR-21* was also detected in various malignant tissues, such as prostate and lung cancer and their metastases, pancreatic cancer and colorectal cancer (17-21). However, in contrast to *miR-1*, *miR-21* appears to be an oncomir. There are preliminary investigations, showing *miR-21* to appear as an oncomir in the case of RCC (22, 23).

The fact that cancer cells have a further survival advantage by extending their chromosomal ends by means of endogenic telomerase, not only raises a problem, but also provides a therapeutic approach in cancer therapy (4).

In order to gain deeper insight into these issues, as far as we are aware, we are the first to investigate one non-malignant

*These Authors contributed equally to this study.

Correspondence to: Matthias Stope, Department of Urology, University Medicine Greifswald, Ferdinand-Sauerbruch-Straße, 17475 Greifswald, Germany. Tel: +49 38348680436, Fax: +49 38348680435, e-mail: matthias.stope@uni-greifswald.de

Key Words: Renal cell carcinoma, microRNA-1, microRNA-21, proliferation.

and four established RCC cell lines regarding their *miR-1* and *miR-21* expression and effect on cell proliferation, additionally determining both telomerase expression and activity.

Materials and Methods

Cell culture. Human RCC cell lines Caki-1, 786-O, A498 (all Cell Lines Service, Eppelheim, Germany), and RCC4 (Sigma-Aldrich, München, Germany) and the non-malignant renal cell line RC-124 (Cell Lines Service) were applied in this study. Caki-1 and A498 cells were propagated in minimum essential medium (MEM) supplemented with 79.6 mg/l non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, 1% penicillline/streptomycin (P/S), and 10% fetal bovine serum (FBS; all PAN Biotech, Aidenbach, Germany), 786-O cells were propagated in RPMI-1640 medium containing 2 mM L-glutamine, 1% P/S, and 10% FBS (all PAN Biotech), RCC4 cells were propagated in Dulbecco's modified Eagle's medium with 1 mM sodium pyruvate, 1% P/S, and 10% FBS (all PAN Biotech), and RC-124 cells were propagated in McCoy's 5a medium supplemented with 2 mM L-glutamine, 1% P/S, and 10% FBS (all PAN Biotech). All cells were passaged twice a week in a humidified atmosphere at 37˚C with 5% CO_2.

Proliferation assay. Cellular proliferation of cells was assessed by cell counting (CASY Cell Counter and Analyzer Model TT; Roche Applied Science, Mannheim, Germany). Adherent cells were detached by trypsin/ethylenediaminetetra-acetic acid treatment, suspended in CASYton (1:100 dilution; Roche Applied Science) and 400 μl of the cell suspension was analyzed in triplicates. Measurement was performed using a capillary of 150 μm in diameter and cell line-specific gate settings to discriminate between living cells, dead cells, and cellular debris: 6.9 μm/14.7 μm for 786-O, 6.5 μm/12.75 μm for RC-124, 8.5 μm/15.75 μm for RCC4, 7.2 μm/15.75 μm for A-498, and 8.57 μm/15.4 μm for Caki-1 cells.

RNA preparation and cDNA synthesis. Total RNA was prepared using peqGOLDTrifast Reagent (Peqlab Biotechnology, Erlangen, Germany) according to the manufacturer's instructions. Subsequently, total RNA concentration was determined utilizing a Nanodrop 2000c UV/vis spectrophotometer (Peqlab Biotechnology) and RNA was stored at −80˚C. To perform the reverse transcription, 100 ng of total RNA were used with Superscript III Reverse Transcriptase (Life Technologies, Darmstadt, Germany) according to the protocol of Chen *et al*. (19) and stem-loop primers as follows: *miR-1* stem-loop: 5'-GTCGTATCCAGTGCAGGG TCCGAG GTATTCGCACTGGATACGACATACAT-3'; *miR-21* stem-loop: 5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATAC GACTCAACA-3'; *U6* stem-loop: 5'-GTCATCCTTGCGCAGG-3'.

Quantification of miR-1 and miR-21 by polymerase chain reaction (PCR). Quantification of microRNAs was performed with SensiMix SYBR hi-ROX Kit (Bioline, Luckenwalde, Germany) on a CFX96 Real-Time System (Bio-Rad, Munich, Germany) with CFX Manager software (Bio-Rad). Sequences of PCR oligos were as follows: *miR-1* forward: 5'-GCCCGCTGGAATGTAAAGAAGT ATG-3'; *miR-21* forward: 5'-GCCCGCTAGCTTATCAGACTG ATG-3'; universal reverse primer (used for *miR-1* and *miR-21* amplification): 5'-GTGCAGGGTCCG AGGT-3'; *U6* forward: 5'-CGCTTCGGCAGCACATATAC-3'; U6 reverse: 5'-AGGGGCCA TGCTAATCTTCT-3'. After one initial denaturation cycle (95˚C for 5 min) 45 amplification cycles were performed (95˚C for 10 s, 60˚C for 20 s, and 72˚C for 10 s), followed by a melting-curve analysis. For quantification, *miR-1* and *miR-21* signals were standardized to *U6* RNA as reference.

Telomerase expression and activity. Cell lines were lysed and RNA preparation was carried out as described above. All RNA samples were subjected to DNAse I (Promega, Madison, WI, USA) treatment. cDNA was synthesized using random hexamers primer and Superscript III Reverse Transcriptase (Life Technologies) according to the manufacturer's instructions. For quantitative analysis of human telomerase reverse transcriptase (*hTERT*) mRNA expression, 60-90 ng cDNA was analyzed using a sequence detector (7500 Fast Real-Time PCR System; Life Technologies) and TaqMan target mixes (Assay-on-Demand Gene expression reagents; Thermo Scientific: ID Hs00972656m1).

For the determination of telomerase activity, TRAPeze RT Telomerase Detection Kit (Merck Millipore, Darmstadt, Germany) was used according to the manufacturer's instructions. Cells were lysed by repeated freeze and thaw cycles in 3-[(3cholamido-propyl)dimethylammonio]-I-propanesulfonate (CHAPS) buffer followed by 30 min of incubation on ice. After centrifugation at 16,000 × *g* for 20 min at 4˚C, aliquots of the supernatant were stored at −80˚C. Protein concentration of extract was determined with DC Protein Assay (Bio-Rad). Using an ABI Prism 7500 Fast real-time cycler (Life Technologies), samples were incubated for 30 min at 30˚C and amplified by PCR (45 cycles: 15 s at 94˚C, 1 min at 59˚C and 10 s at 45˚C). The threshold cycle values (c_t) were determined from semi-log amplification plots (log increase in fluorescence *versus* cycle number) and compared with standard curves generated from standard telomeric repeats provided with the kit.

Statistics. For data evaluation, the graphics and statistical software Graph Pad Prism v. 5.01 (GraphPad Software, La Jolla, CA, USA) was used. Statistical analyses were performed by using the unpaired Student's *t*-test, with acceptance of significance at $p \leq 0.05$.

Results

The non-malignant cell line RC-124 was established from primary cell preparations of non-tumor kidney tissue of a male carcinoma patient in 1998. Although frequently used as a non-malignant control, nothing is known about the experimental modality of cell immortalization which resulted in the RC-124 cell line. Since telomerase-transduced immortalization is often used for the generation of cell lines, we examined telomerase expression and activity in RC-124 cells compared to malignant RCC cell lines.

Notably, non-malignant RC-124 cells clearly transcribed the telomerase catalytic subunit *hTERT* mRNA (Figure 1A) accompanied by pronounced enzymatic activity (Figure 1B). Regarding the four malignant cell lines Caki-1, 786-O, RCC4 and A498, telomerase expression and activity was explicitly detectable, as expected for cancer cells. What is striking here is the fact that telomerase expression and activity in highly proliferative Caki-1 and 786-O cells was significantly higher than in the poorly proliferative RCC4,

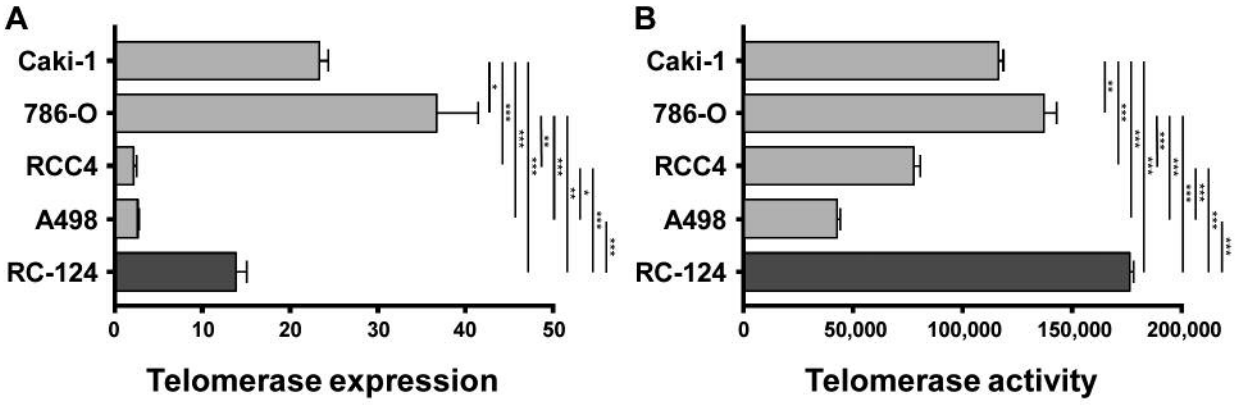

Figure 1. *Presence of telomerase expression and activity. A: Human telomerase reverse transcriptase (hTERT) mRNA was quantified by reverse transcriptase-polymerase chain reaction. B: Telomerase activity was quantified applying the TRAPeze RT Telomerase Detection Kit (Merck Millipore, Darmstadt, Germany). Results are expressed as the mean±SD. Significantly different at *p≤0.05, **p≤0.01 and ***p≤0.001.*

and A498 cells. Compared to the four malignant cell lines, non-malignant RC-124 cells displayed relatively high expression of *hTERT* and very high telomerase activity.

Within the scope of our investigations, we examined the cellular growth of the non-malignant renal cell line RC-124 compared to the four RCC cell lines. As shown in Figure 2, the malignant cell lines Caki-1, 786-O, RCC4 and A498 have a different growth pattern compared to the non-malignant RC-124 cell line. Furthermore, even within the malignant cell lines, inconsistencies were found. The two malignant cell lines Caki-1 and 786-O exhibited higher proliferation compared to RC-124, whereas RCC4 and A498 cells exhibited a markedly lower increase of proliferation within 120 h, even lower than that of non-malignant RC-124 cells, in spite of being malignant. The strikingly different growth characteristics, especially the subdued growth of malignant cell lines A498 and RCC4, prompted us to investigate whether two different microRNAs influence the described findings.

Figure 3 shows the expression of the microRNAs *miR-1* and *miR-21* in the non-malignant and the four malignant cell lines. For Caki-1, as a highly proliferative cell line, the expression of *miR-1,* was not only weaker compared to the non-malignant RC-124 cells, but in fact was also the weakest in comparison to all other cell lines. In order of streangth, *miR-1* expression in RCC-4 cells followed that of Caki-1 cells. It is striking that Caki-1, as a highly proliferative cell line in our studies, exhibited a distinctly opposite expression of *miR-1* compared to A498, a cell line with reduced proliferation compared to non-malignant RC-124 cells: expression was low in the case of Caki-1 cells but high in the case of A498 cells. However, regarding the expression of *miR-21* in Caki-1 and RCC-4 cells, a contrasting expression pattern was observed. As already recognized in the proliferative behavior, Caki-1 cells lead in the expression of *miR-21*. In general, the differences in *miR-21* expression between cell lines are not as great as in the expression of *miR-1*.

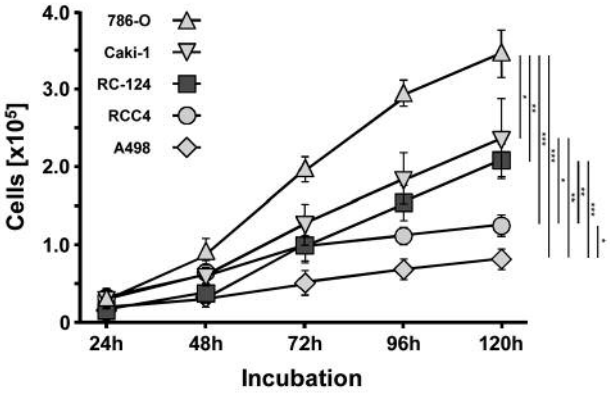

Figure 2. *Cell growth pattern of renal cell cancer cells Caki-1, 786-O, RCC-4, and A498 compared to the non-malignant renal cells RC-124. Cells were counted using a CASY Cell Counter and Analyzer Modell TT (Roche Applied Science, Mannheim, Germany) at the indicated time points. Results are expressed as the mean±SD. Significantly different at *p≤0.05, **p≤0.01 and ***p≤0.001.*

Discussion

As RCC is the most common type of kidney cancer, with an increasing incidence (7, 24), it is important to explore and understand the tumor biology of this malignancy in order to find new early diagnostic markers and therapeutic approaches. Cancer cells aquire various mechanisms by which they escape apoptosis, become immortal, or provide to them at least a survival advantage compared to non-malignant

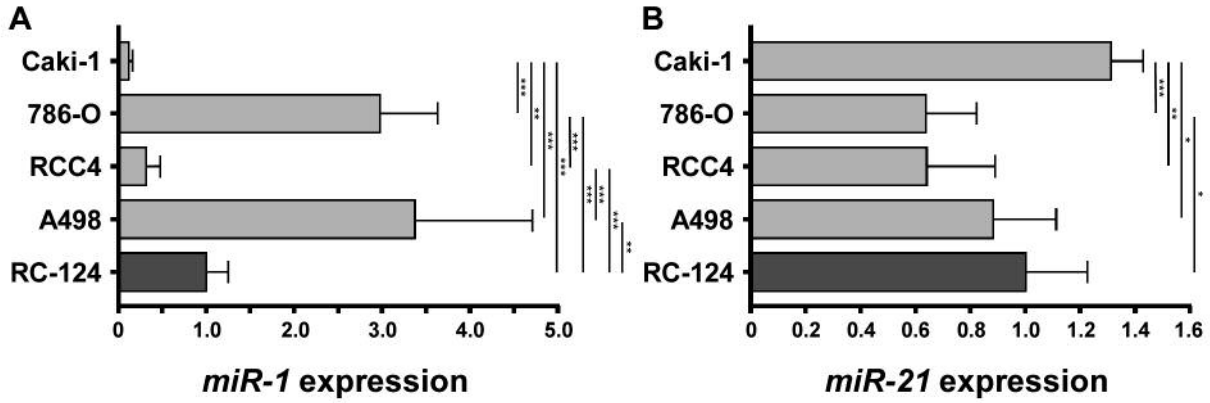

Figure 3. *Expression of the microRNAs miR-1 (A) and miR-21 (B) as quantified by reverse transcriptase-polymerase chain reaction. Results are expressed as the mean±SD. Significantly different at *p≤0.05, **p≤0.01 and ***p≤0.001.*

cells (25). Thus, tumor formation is mediated by ensuring adequate blood supply through the tumor cells themselves, by initiating angiogenesis (2), immunomodulation (3) and the attainment of immortality, not only by escaping apoptosis, but also by the presence of telomerase (4).

One strategy tumor cells use to hijack and reprogram regulatory pathways is the modulation of microRNAs, acting as tumor suppressors or oncogenes depending on tumor entity, and consequently playing a role in carcinogenesis. After their first discovery in the 1990s, focus was particularly on the ability of microRNAs to regulate gene expression, especially with regard to cancer (7, 26). MicroRNAs are a class of small non-coding RNAs which can interfere with mRNA and are able to regulate gene expression at the post-transcriptional level (7). Several studies have dealt with the effects of different microRNAs in RCC, such as *miR-21*, but not *miR-1*. Cell proliferative properties of *miR-1* have been investigated in various tumor tissues, such as ovarian, lung, colorectal, prostate, bladder and endometrial cancer, as well as RCC (14, 27-32).

However, the thesis that *miR-1* functions as a tumor suppressor, supported by the above-mentioned investigations, cannot be regarded as being set in stone. In context of a study on gastric cancer, *miR-1* was shown to be associated with a higher incidence of liver metastases (33). This finding was confirmed by two studies on ovarian carcinoma cells, which also provided initial indications that *miR-1* can lose its role as a classic tumor suppressor (27, 34). Although the former studies dealt with other malignant entities, a similar situation is also apparent for RCC within the framework of the present investigations. Unfortunately, to the best of our knowledge, there are only two investigations regarding *miR-1* expression in RCC (32, 35), describing miR-1 as a tumor suppressor, by targeting transgelin-2 mRNA (32). Our results indicate a more complex regulation and may point to some individual aspects of *miR-1* functionality in RCC development. In the case of RCC cell lines Caki-1 and A498, *miR-1* appears to function as a tumor suppressor. In Caki-1 cells, a low level of *miR-1* was accompanied by a high rate of proliferation, whereas the explicitly elevated *miR-1* level in A498 cells led to attenuated cellular growth. In contrast to this, *miR-1* in 786-O and RCC4 cells appeared to be dysregulated regarding the proliferative capacity. Even though *miR-1* is strongly expressed in 786-O cells, the cell line was found to exhibit rather highly proliferative behavior comparable to that of Caki-1 cells. In contrast, while RCC4 cells demonstrated a very low expression of *miR-1* out of the panel of RCC cell lines, RCC4 proliferation was very low.

Our findings confirm previous work showing *miR-2* is pro-oncogenic in different malignancies. Thus, in all tested RCC cell lines, as well as in the non-malignant RC-124 cell line, *miR-21* expression was relatively similar. The suspected role of *miR-21* as an oncomir is also confirmed by the proliferative behavior of Caki-1 cells, with their having the strongest proliferation and *miR-21* expression in our analyses. Liu *et al*. reported that the expression of *miR-21* in RCC was not only associated with a poor prognosis, but also had an impact on recurrence. They found that expression of *miR-21* was greater in malignant A498 cells compared to the kidney cell line HRPTEpiC and *miR-21* suppresses the p53/p21-cyclin E2-B-cell-associated X protein/caspase-3 signaling pathway (36).

Malignant A498 cells did not show higher *miR-21* expression in our study compared to that of the non-malignant cells, however, the differences in expression were not very pronounced. This might be because of our selection of another cell line for control cells, RC-124 rather than HRPTEpiC. In a review by Gu *et al*., *miR-21* was shown to

be a promising marker and therapeutic target in RCC (7). It is also known that *miR-21* drives RCC cell proliferation and invasion by mediating post-transcriptional regulation of phosphatase and tensin homolog that in turn increased an oncogenetic pathway (7, 37). In addition, the post-transcriptional down-regulation of the programmed cell death protein 4 tumor-suppressor gene by *miR-21* is another example of *miR-21* acting as an oncomir (38).

The possibility that cancer cells can proliferate unhindered is not only due to the presence of microRNA, but also due to the fact that cancer cells have the ability to prevent the degradation of their chromosomal ends. Telomeres are the tails of the chromosomes, and are variable in length. Human telomeres, with a length of about 10 kb, are shortened at each cell division. If telomeres reach a length of 4 kb after approximately 50-100 cell divisions, this is associated with a transition of the cell into the resting phase of the cell cycle (4) and a shrinking of chromosomal ends (39). This in turn means that the cell cannot divide anymore.

Telomerase, a reverse transcriptase that counteracts telomere degradation by restoration, gives the cell a longer or infinite lifespan (40). In humans, telomerase activity is low in or absent from the majority of cells (4). In contrast to non-malignant cells, however, cancer cells have a high telomerase activity, which generates a further survival advantage – they are able to divide infinitely (40). In accordance with the detailed knowledge about the role of telomerases in cancer cells, it is not surprising that our analyses have shown that in highly proliferative RCC cell lines, Caki-1 and 786-O, both the expression of telomerase and its activity were particularly pronounced, in contrast to the more weakly proliferative RCC4 and A498 cells. The strong telomerase expression in the non-malignant RC-124 cells, however, was certainly provoked by genetic engineering with the aim of generating an immortalized cell line.

All in all, it can be seen that our finding, namely *miR-21* functioning as an oncomir, have been reported in past studies (7, 36-38). Additionally, we were able to show that not only in ovarian cancer (27, 34), but also in RCC, the facade of *miR-1* as a classic tumor suppressor continues to crumble. Due to the fact that the exact underlying mechanisms in RCC are not yet cataloged, comprehensive research is necessary to establish *miR-1* as well as *miR-21* in tumor cells as a new prognostic marker in RCC.

Conclusion

As far as we know, this is the first study to compare *miR-1* and *miR-21* in terms of their expression and their proliferative capacities in four established RCC cell lines compared to a non-malignant renal cell line. Notably, *miR-1* and *miR-21* functionality in cancer progression may be more dependent on the individual cellular context, *e.g.* cancer stage, as expected. These results may question the general classification system of microRNAs as bearing anti-oncogenic or oncogenic cellular functions in all stages of all malignancy. Additionally, and most likely due to immortalization, non-malignant RC-124 cells exhibited telomerase expression and activity.

miR-1 and *miR-21* may be also useful as biomarkers for tumor heterogeneity and, in combination with other stratifying biomarkers, for RCC diagnosis, prognosis, or treatment response.

Competing Interests

The Authors declare that they have no competing interests.

Acknowledgements

The Authors thank Katja Wittig for excellent technical assistance and are grateful to Rüdiger Bode for fruitful discussion.

References

1 Parihar JS and Tunuguntla HSGR: Role of chemokines in renal cell carcinoma. Rev Urol *16*: 118-121, 2014.
2 Dosquet C, Coudert MC, Lepage E, Cabane J and Richard F: Are angiogenic factors, cytokines, and soluble adhesion molecules prognostic factors in patients with renal cell carcinoma? Clin Cancer Res *3*: 2451-2458, 1997.
3 Lahn M, Fisch P, Köhler G, Kunzmann R, Hentrich I, Jesuiter H, Behringer D, Muschal B, Veelken H, Kulmburg P, Ikle DN and Lindemann A: Pro-inflammatory and T-cell-inhibitory cytokines are secreted at high levels in tumor cell cultures of human renal cell carcinoma. Eur Urol *35*: 70-80, 1999.
4 Osterhage JL and Friedman KL: Chromosome end maintenance by telomerase. J Biol Chem *284*: 16061-16065, 2009.
5 Low G, Huang G, Fu W, Moloo Z and Girgis S: Review of renal cell carcinoma and its common subtypes in radiology. World J Radiol *8*: 484-500, 2016.
6 Xu Q, Krause M, Samoylenko A , and Vainio S: Wnt signaling in renal cell carcinoma. Cancers (Basel) *8*: E57, 2016.
7 Gu L, Li H, Chen L, Ma X, Gao Y, Li X, Zhang Y, Fan Y and Zhang X: MicroRNAs as prognostic molecular signatures in renal cell carcinoma: a systematic review and meta-analysis. Oncotarget *6*: 32545-32560, 2015.
8 Li M, Wang Y, Song Y, Bu R, Yin B, Fei X, Guo Q and Wu B: MicroRNAs in renal cell carcinoma: a systematic review of clinical implications (Review). Oncol Rep *33*: 1571-1578, 2015.
9 Acunzo M, Romano G, Wernicke D and Croce CM: MicroRNA and cancer–a brief overview. Adv Biol Regul 57: 1-9, 2015.
10 Baranwal S and Alahari SK: miRNA control of tumor cell invasion and metastasis. Int J Cancer *126*: 1283-1290, 2010.
11 Hwang HW and Mendell JT: MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer *94*: 776-780, 2006.
12 Chen J, Yin H, Jiang Y, Radhakrishnan SK, Huang ZP, Li J, Shi Z, Kilsdonk EP, Gui Y, Wang DZ and Zheng XL: Induction of *microRNA-1* by myocardin in smooth muscle cells inhibits cell proliferation. Arterioscler Thromb Vasc Biol *31*: 368-375, 2011.

13 Minemura H, Takagi K, Miki Y, Shibahara Y, Nakagawa S, Ebata A, Watanabe M, Ishida T, Sasano H and Suzuki T: Abnormal expression of *miR-1* in breast carcinoma as a potent prognostic factor. Cancer Sci *106*: 1642-1650, 2015.
14 Stope MB, Stender C, Schubert T, Peters S, Weiss M, Ziegler P, Zimmermann U, Walther R and Burchardt M: Heat-shock protein HSPB1 attenuates microRNA *miR-1* expression thereby restoring oncogenic pathways in prostate cancer cells. Anticancer Res *34*: 3475-3480, 2014.
15 Kanaan Z, Rai SN, Eichenberger MR, Roberts H, Keskey B, Pan J and Galandiuk S: Plasma *miR-21*: a potential diagnostic marker of colorectal cancer. Ann Surg *256*: 544-551, 2012.
16 Weiss M, Brandenburg LO, Burchardt M and Stope MB: *MicroRNA-1* properties in cancer regulatory networks and tumor biology. Crit Rev Oncol Hematol *104*: 71-77, 2016.
17 Yang Y, Guo JX and Shao ZQ: miR-21 targets and inhibits tumor suppressor gene *PTEN* to promote prostate cancer cell proliferation and invasion: an experimental study. Asian Pac J Trop Med *10*: 87-91, 2017.
18 Zhao W, Zhao JJ, Zhang L, Xu QF, Zhao YM, Shi XY and Xu AG: Serum *miR-21* level: a potential diagnostic and prognostic biomarker for non-small cell lung cancer. Int J Clin Exp Med *8*: 14759-14763, 2015.
19 Dong J, Zhang Z, Gu T, Xu SF, Dong LX, Li X, Fu BH and Fu ZZ: The role of *microRNA-21* in predicting brain metastases from non-small cell lung cancer. Onco Targets Ther *10*: 185-194, 2017.
20 Abue M, Yokoyama M, Shibuya R, Tamai K, Yamaguchi K, Sato I, Tanaka N, Hamada S, Shimosegawa T, Sugamura K and Satoh K: Circulating *miR-483-3p* and *miR-21* is highly expressed in plasma of pancreatic cancer. Int J Oncol *46*: 539-547, 2015.
21 Mima K, Nishihara R, Yang J, Dou R, Masugi Y, Shi Y, da Silva A, Cao Y, Song M, Nowak J, Gu M, Li W, Morikawa T, Zhang X, Wu K, Baba H, Giovannucci EL, Meyerhardt JA, Chan AT, Fuchs CS, Qian ZR and Ogino S: MicroRNA *MIR21* (miR-21) and PTGS2 expression in colorectal cancer and patient survival. Clin Cancer Res *22*: 3841-3848, 2016.
22 Cao J, Liu J, Xu R, Zhu X, Liu L and Zhao X: *MicroRNA-21* stimulates epithelial-to-mesenchymal transition and tumorigenesis in clear cell renal cells. Mol Med Rep *13*: 75-82, 2016.
23 Cancer Genome Atlas Research Network: Comprehensive molecular characterization of clear cell renal cell carcinoma. Nature *499*: 43-49, 2013.
24 Lipworth L, Tarone RE and McLaughlin JK: The epidemiology of renal cell carcinoma. J Urol *176*: 2353-2358, 2006.
25 Hanahan D and Weinberg RA: Hallmarks of cancer: the next generation. Cell *144*: 646-674, 2011.
26 Lee RC, Feinbaum RL and Ambros V: The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. Cell *75*: 843-854, 1993.
27 Stope MB, Hettenbach D, Kaul A, Paditz M, Diesing K, Burchardt M, Zygmunt M, Mustea A and Koensgen D: The tumor suppressor *microRNA-1* exhibits restricted inhibition of proliferation of ovarian cancer cells. Anticancer Res *36*: 3329-3334, 2016.
28 Nasser MW, Datta J, Nuovo G, Kutay H, Motiwala T, Majumder S, Wang B, Suster S, Jacob ST and Ghoshal K: Down-regulation of micro-RNA-1 (*miR-1*) in lung cancer. Suppression of tumorigenic property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by *miR-1*. J Biol Chem 283: 33394-33405, 2008.
29 Reid JF, Sokolova V, Zoni E, Lampis A, Pizzamiglio S, Bertan C, Zanutto S, Perrone F, Camerini T, Gallino G, Verderio P, Leo E, Pilotti S, Gariboldi M and Pierotti MA: miRNA profiling in colorectal cancer highlights miR-1 involvement in MET-dependent proliferation. Mol Cancer Res *10*: 504-515, 2012.
30 Yoshino H, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Nishiyama K, Nohata N, Seki N and Nakagawa M: The tumour-suppressive function of *miR-1* and *miR-133a* targeting *TAGLN2* in bladder cancer. Br J Cancer *104*: 808-818, 2011.
31 Xiao H, Zeng J, Li H, Chen K, Yu G, Hu J, Tang K, Zhou H, Huang Q, Li A, Li Y, Ye Z, Wang J and Xu H: *miR-1* down-regulation correlates with poor survival in clear cell renal cell carcinoma where it interferes with cell cycle regulation and metastasis. Oncotarget *30*: 13201-13215, 2015.
32 Kawakami K, Enokida H, Chiyomaru T, Tatarano S, Yoshino H, Kagara I, Gotanda T, Tachiwada T, Nishiyama K, Nohata N, Seki N and Nakagawa M: The functional significance of *miR-1* and *miR-133a* in renal cell carcinoma. Eur J Cancer *48*: 827-836, 2012.
33 Huang D, Wang H, Liu R, Li H, Ge S, Bai M, Deng T, Yao G and Ba Y: *miRNA27a* is a biomarker for predicting chemosensitivity and prognosis in metastatic or recurrent gastric cancer. J Cell Biochem *115*: 549-556, 2014.
34 Stope MB, Delogu S, Diesing K, Klinkmann G, Evert M, Koensgen D, Zygmunt M, Burchardt M and Mustea A: Expression pattern of the microRNA *miR-1* in ovarian cancer cell lines and tumor tissue samples implies a loss of miR-1's tumor-suppressor properties. RNA Dis *1*: 1-6, 2014.
35 Hidaka H, Seki N, Yoshino H, Yamasaki T, Yamada Y, Nohata N, Fuse M, Nakagawa M and Enokida H: Tumor suppressive *microRNA-1285* regulates novel molecular targets: aberrant expression and functional significance in renal cell carcinoma. Oncotarget *3*: 44-57, 2012.
36 Liu Z, Lu Y, Xiao Y and Lu Y: Up-regulation of miR-21 expression is a valuable predicator of advanced clinicopathological features and poor prognosis in patients with renal cell carcinoma through the p53/p21-cyclin E2-BAX/caspase-3 signaling pathway. Oncol Rep *37*: 1437-1444, 2017.
37 Dey N, Das F, Ghosh-Choudhury N, Mandal CC, Parekh DJ, Block K, Kasinath BS, Abboud HE and Choudhury GG: *microRNA-21* governs TORC1 activation in renal cancer cell proliferation and invasion. PLoS One *7*: e37366, 2012.
38 Li X, Xin S, He Z, Che X, Wang J, Xiao X, Chen J and Song X: MicroRNA-21 (*miR-21*) post-transcriptionally down-regulates tumor suppressor *PDCD4* and promotes cell transformation, proliferation, and metastasis in renal cell carcinoma. Cell Physiol Biochem *33*: 1631-1642, 2014.
39 Wright WE, Piatyszek MA, Rainey WE, Byrd W and Shay JW: Telomerase activity in human germline and embryonic tissues and cells. Dev Genet *18*: 173-179, 1996.
40 Cech TR: Beginning to understand the end of the chromosome. Cell *116*: 273-279, 2004.

Received April 1, 2017
Revised April 24, 2017
Accepted April 27, 2017