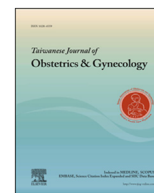
Taiwanese Journal of Obstetrics & Gynecology 53 (2014) 536–541

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com



Original Article

Serum microRNAs in clear cell carcinoma of the ovary

Angel Chao [a, b], Chyong-Huey Lai [a, b], Hua-Chien Chen [c, d], Chiao-Yun Lin [a, b], Chia-Lung Tsai [a, e], Yun-Hsin Tang [a, b], Huei-Jean Huang [a, b], Chen-Tao Lin [a, b], Min-Yu Chen [a, b], Kuang-Gen Huang [a, b], Hung-Hsueh Chou [a, b], Ting-Chang Chang [a, b], Shu-Jen Chen [c, d, **], Tzu-Hao Wang [a, b, e, f, *]

[a] Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[b] Gynecologic Cancer Research Centre, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[c] Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan
[d] Department of Biomedical Science, School of Medicine, Chang Gung University, Taoyuan, Taiwan
[e] Genomic Medicine Research Core Laboratory, Chang Gung Memorial Hospital, Taoyuan, Taiwan
[f] School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan

ARTICLE INFO

Article history:
Accepted 14 July 2014

Keywords:
clear cell carcinoma
hsa-miR-130a
microRNA
ovarian cancer

ABSTRACT

Objective: To identify candidate microRNAs (miRNAs) in the serum of patients with clear cell carcinomas in monitoring disease progression.
Materials and methods: The sera of patients with diagnosed ovarian clear cell carcinoma were collected from 2009 to 2012. Real-time quantitative polymerase chain reaction (PCR) analysis for 270 miRNAs was performed. To offset the potential extraction bias, an equal amount of *Caenorhabditis elegans* cel-miR-238 was added to each serum specimen before miRNA isolation. miRNA expression was analyzed using the ΔCt method, with cel-miR-238 as controls.
Results: Twenty-one patients with clear cell carcinoma were included. In the discovery phase on four pairs of pre- and postoperative sera, 18 differentially expressed miRNAs were selected from 270 miRNAs. In the validation phase on an independent set of 11 pairs of pre- and postoperative sera, 4 miRNAs (hsa-miR-130a, hsa-miR-138, hsa-miR-187, and hsa-miR-202) were confirmed to be higher in the preoperative sera. In the application phase, hsa-miR-130a remained consistent with the different time points in seven of the 10 patients during clinical follow-up periods. More importantly, in three patients, hsa-miR-130a levels were elevated in early disease recurrences before CA125 was found to be elevated.
Conclusion: Hsa-miR-130a may be a useful serum biomarker for detecting recurrence of ovarian clear cell cancer, and warrants further studies.
Copyright © 2014, Taiwan Association of Obstetrics & Gynecology. Published by Elsevier Taiwan LLC. All rights reserved.

Introduction

Epithelial ovarian cancers are one of the most lethal gynecologic malignancies [1]. Recent discoveries have revealed that epithelial ovarian cancer is a heterogeneous disease [2–4]. Clear cell carcinoma is a distinct disease with an increased prevalence reported in Asian ethnic groups, such as in Taiwan [5] and Japan [6]. Clear cell carcinomas are more resistant to the standard chemotherapy of platinum and paclitaxel than to its serous counterparts in the advanced stages [7]. Clear cell carcinoma of the ovary has been reported to be associated with endometriosis [4] and the ARID1A mutation [8]. However, the tumorigenesis of ovarian clear cell cancer is still unclear, and clinical management of this disease remains challenging.

MicroRNAs (miRNAs) are noncoding RNA molecules that are 21–25 nucleotides in length, and bind to the 3′-untranslated regions of mRNAs where they inhibit protein translation or promote mRNA degradation [9]. Many miRNAs are evolutionarily conserved across plants and animals, indicating that they are involved in a number of diverse processes. In human cancers, miRNAs may act as oncomirs or tumor suppressors [10]. Because distinct miRNA signatures in

* Corresponding author. Department of Obstetrics and Gynecology, Chang Gung Memorial Hospital, 5 Fu-Hsing Street, Gwei-Shan, Taoyuan, Taiwan.
** Corresponding author. Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan.
E-mail addresses: sjchen@mail.cgu.edu.tw (S.-J. Chen), knoxtn@cgmh.org.tw (T.-H. Wang).

http://dx.doi.org/10.1016/j.tjog.2014.07.005
1028-4559/Copyright © 2014, Taiwan Association of Obstetrics & Gynecology. Published by Elsevier Taiwan LLC. All rights reserved.

chronic lymphocytic leukemia were reported [11], cancer-specific miRNA profiles have been used to classify the origin of solid tumors [12]. The roles of miRNAs in cancer tissues, including ovarian cancer, have been demonstrated in numerous reports [13–15]. In recent years, circulating miRNAs in serum or plasma have been proposed as possible biomarkers for specific cancers [16–19]. In this study, we aimed to identify candidate miRNAs to be used in monitoring the clinical course of clear cell carcinoma.

Materials and methods

Patients

The sera of patients with diagnosed ovarian clear cell carcinomas were collected from 2009 to 2013 in our hospital. These patients included newly diagnosed cases and those who were undergoing follow up. Specimens with obvious hemolysis were excluded. The Institute Review Board of Chang Gung Memorial Hospital approved this study (CGMH-IRB #99-0112B and #98-3644B).

Serum RNA extraction and reverse transcription

Total RNA was isolated using QIAzol reagent (QIAGEN, Taipei, Taiwan) and miRNeasy Mini Kit (QIAGEN). Briefly, 300 μL of serum sample was mixed with 700 μL of QIAzol reagent and incubated at room temperature for 5 minutes. One microliter of 0.05 nM synthetic *Caenorhabditis elegans*-specific microRNA cel-miR-238 was added to each serum specimen as the spike-in control. RNA was purified following the manufacture's protocol and eluted in 30 μL of RNase-free water. Similar procedures, as previously described [20,21], were performed to convert all miRNAs into corresponding complementary DNAs (cDNAs) in one reverse transcription (RT) reaction. Briefly, a 10-μL reaction mixture containing miRNA-specific stem-loop RT primers (final 0.1 μM each), 500 μM of deoxy-ribonucleoside triphosphate (dNTP) (final), 1 μL of Dithiothreitol (DTT), 0.5 μL of RNase out, 2 μL of 5 × FS buffer, 0.5 μL of Superscript III (Invitrogen, Carlsbad, CA, USA), and 4.5 μL of total RNA were used for the RT reaction. The pulsed RT reaction was performed as follows: 16°C for 30 minutes, followed by 50 cycles at 20°C for 30 seconds, 42°C for 30 seconds, and 50°C for 1 second. The RT product was diluted 20-fold and stored at −80°C.

Quantitative RT-(RT-qPCR) polymerase chain reaction

For miRNA quantification, 1 μL of diluted RT product was used as template for a 10-μL polymerase chain reaction (PCR). Briefly, 1 × SYBR/TaqMan Master Mix (Applied Biosystem, Foster City, CA, USA), 200 nM of miRNA-specific forward primer, and 200 nM of universal reverse primer were used for each PCR assay. The thermocycles for Q-PCR were: 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 63°C for 32 seconds, and a dissociation stage. All reactions were performed using the ABI Prism 7900 Fast Real-Time PCR system (Applied Biosystem).

Data analysis

miRNA expression level was analyzed using the ΔCt method, with cel-miR-238 as controls. After normalizing the cel-miR-238 level in each sample, expression levels of individual miRNAs were presented as 39 substrate the value of threshold cycle (Ct) that was defined as the cycle number when SYBR green/TaqMan signals appeared. Nonparametric, Wilcoxon tests and Mann–Whitney U tests were performed to identify significantly differential expression of miRNAs between preoperative and postoperative sera.

Results

Screening 270 miRNAs in the discovery phase

A total of 25 patients with clear cell carcinoma were included in this study (Fig. 1). The median age of the patients was 49 years old (range, 32–59 years). The patients were of International Federation of Gynecology and Obstetrics (FIGO) stage IC ($n = 17$), stage IIB ($n = 1$), IIC ($n = 1$), IIIC ($n = 2$), IV ($n = 3$), and stage X ($n = 1$). Four of the 25 patients also had endometriosis: two cases in the validation phase and two in the application phase (Fig. 1). Four pairs of pre- and postoperative sera were used to select the differentially expressed miRNAs from 270 miRNAs that have been characterized in our laboratory. Eighteen miRNAs exhibited changes between pre- and postoperative sera that were >1.25 fold (Table 1) and were thus further analyzed in the validation phase.

Identification of serum miRNA as potential biomarker

In the validation phase on 11 pairs of sera (Fig. 1), the levels of four miRNAs, namely hsa-miR-130a, hsa-miR-138, hsa-miR-187, and hsa-miR-202, were shown to be higher in the preoperative sera than in the postoperative sera using the SYBR green system (Fig. 2). The primer sequences of the four miRNAs are summarized in Table 2. To convert the SYBR green system to the TaqMan system, we then tested hsa-miR-130a in 12 pairs of preoperative and postoperative sera. A drop in the postoperative hsa-miR-130a was confirmed in nine pairs of sera.

Hsa-miR-130a may be useful for early detection of disease recurrence

In the application phase (Fig. 1), we measured hsa-miR-130a in serum specimens collected from four patients with cancer recurrence (Fig. 3A) and six patients without recurrences (Fig. 3B). The changes in hsa-miR-130a were consistent with disease status in seven patients: increasing trends of hsa-miR-130a were seen in all four patients with recurrent cancer (Fig. 3A), and decreasing trends of hsa-miR-130a in three (50%) out of six patients without recurrence (Fig. 3B). CA125 levels were also measured in all of the specimens. Of note, all of the six patients without recurrent cancer exhibited decreasing trends of serum CA125 levels (Fig. 3B).

The most challenging, but clinically important, task was to identify the cases in which cancer had either already recurred or will recur. In the four patients with cancer recurrence, an increase in CA125 identified only one case (25%), whereas hsa-miR-130a

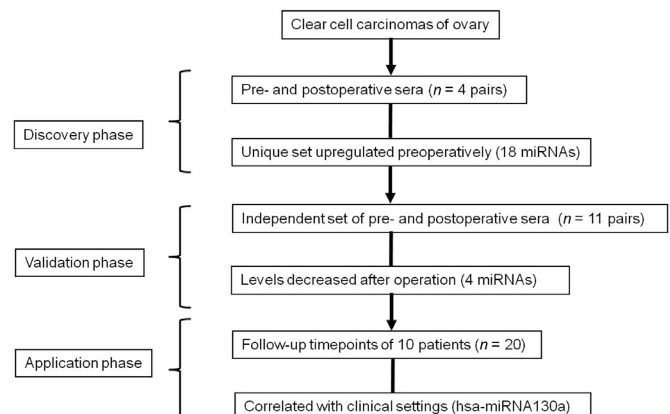


Fig. 1. Study design and analytical steps of this study.

Table 1
MicroRNAs significantly upregulated in the serum of clear cell carcinomas of the ovary (fold change >1.25 and $p < 0.05$).

MicroRNA	Chromosome	Fold change	Mann–Whitney
hsa-miR-202	Chr10_15	2.33	0.03
hsa-miR-365	Chr16_03	2.11	0.03
hsa-miR-1	Chr20_08	2.09	0.03
hsa-miR-130a	Chr11_06	2.07	0.03
hsa-miR-363	ChrX_13	1.93	0.03
hsa-miR-187	Chr18_02	1.91	0.03
hsa-miR-200b	Chr1_01	1.89	0.03
hsa-miR-181a	Chr1_25	1.68	0.03
hsa-miR-138	Chr3_05	1.64	0.03
hsa-miR-29b-1*	Chr7_16	1.55	0.03
hsa-miR-29b	Chr7_16	1.54	0.03
hsa-miR-519d	Chr19_18	1.53	0.03
hsa-miR-195	Chr17_03	1.51	0.03
hsa-miR-25*	Chr7_10	1.43	0.03
hsa-miR-760	Chr1_10	1.39	0.03
hsa-miR-565	Chr3_07	1.38	0.03
hsa-miR-196a*	Chr17_15	1.36	0.03
hsa-miR-125b	Chr11_14	1.27	0.03

Asterisks indicate the historical nomenclature, and the current names are as follows: miR-29b-1* = miR-29b-1-5p; miR-25* = miR-25-5p; miR-196a* = miR-196a-3p.

increased in all of the recurrent cancer cases (100%). More importantly, three cases showed increasing trends of serum hsa-miR-130a even when CA125 levels remained within normal range (≤35 U/mL; Cases 1–3. Fig. 3A).

The clinical histories of Cases 1–3 are summarized as follows (Fig. 3A). For Patient 1 (51 years old, stage IIB), CA125 levels were within normal range in the two sera collected 3 months apart. However, recurrent cancer in the left pouch of Douglas extending to the left vaginal stump was confirmed on computed tomography (CT) 1 month after the first time point. Patient 2 (32 years old, stage IC) underwent the third surgery for multiple peritoneal metastases. CA125 did not increase 6 weeks before the surgery, but hsa-miR-130a was increased between the two time points. For Patient 3 (42 years old, stage IC), tumor metastases were suspected on CT images 3 months after a cytoreductive surgery, but the serum CA125 levels decreased from 9.6 U/mL to 7.1 U/mL in the following 4 months. Advanced intraabdominal cancer recurrences in the mesentery, left paracolic gutter, and subphrenic and hepatorenal space were confirmed intraoperatively 10 months later. Collectively, these results indicate that an elevation of hsa-miR-130a may detect early disease recurrence before an increase in CA125 is seen.

Discussion

Clinical values of circulating miRNAs in blood have been hypothesized to be applicable as a tumor marker in various cancers [19,22–28], and circulating miRNAs have been reported in patients with ovarian cancer [18,29–33]. Those studies were primarily focused on the identification of miRNAs that could be used to follow the serous type of ovarian cancer. To our knowledge, this study is the first report of analyzing serum miRNA panels in patients with ovarian clear cell carcinomas. Our systematic approach has led to the finding that hsa-miR-130a may be a useful marker for early detection of disease recurrence.

CA125 assessment is currently the standard of care in making diagnosis, following response to treatment, and predicting prognosis in ovarian cancer [34]. The sensitivity of CA125 is 85% using the cut-off of 35 U/mL [35]. In our previous report, 12% of invasive ovarian cancers in 403 cases collected between 2000 and 2005 were below normal range [36]. For these patients, biomarkers other than serum CA125 are needed.

Epidemiological and molecular studies have shown strong associations between ovarian clear cell carcinoma and endometriosis [37,38]. In this study, four of the 25 patients also had endometriosis in the final pathology. The plasma miRNAs that have been associated with endometriosis include miR-17-5p, miR-20a, and miR-22 [39]. MiR-199a, miR-122, miR-145, and miR-542-3p have also been proposed to be potential biomarkers for

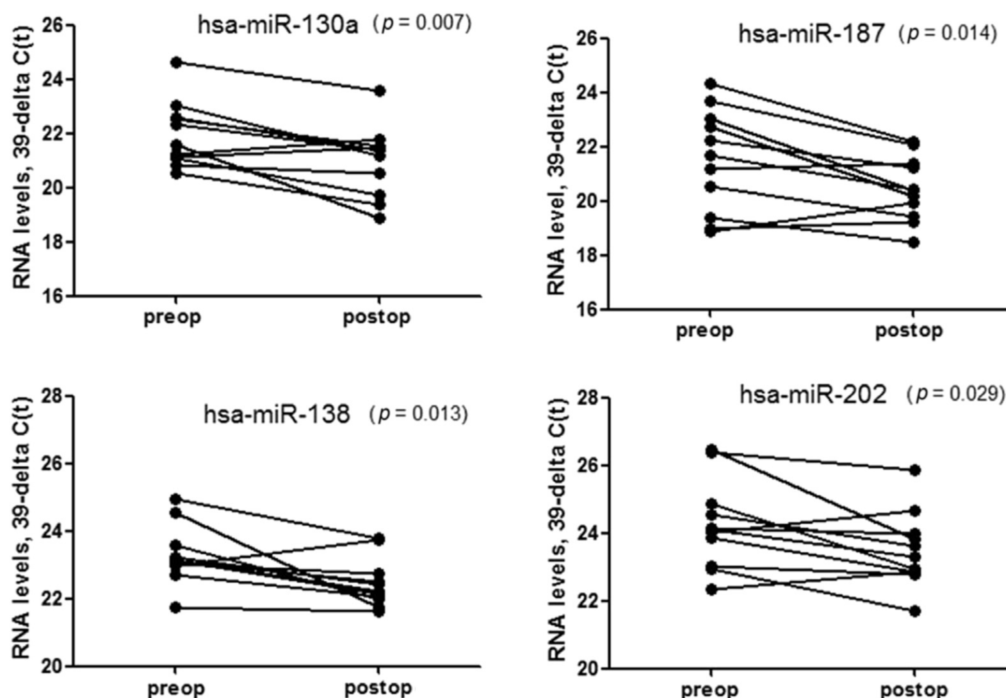


Fig. 2. Four circulating miRNAs were significantly higher in pre- than in postoperative sera. postop = postoperative; preop = preoperative.

Table 2
Primer sequence of the four microRNAs.

microRNA	Sequence
hsa-miR-130a RT primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATGCCCTT
hsa-miR-138 RT primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCGGCCTGA
hsa-miR-187 RT primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCGGCTGC
hsa-miR-202 RT primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGTTCCCATG
hsa-miR-130a qPCR primer	CGGCGGCAGTGCAATGTTAAAA
hsa-miR-138 qPCR primer	CGGCGGAGCTGGTGTTGTGAAT
hsa-miR-187 qPCR primer	CGGCGGTCGTGTCTTGTGTTGC
hsa-miR-202 qPCR primer	CGGCGGAGAGGTATAGGGCATG
Universal qPCR reverse primer	CAACTGGTGTCGTGGAGTCGG

PCR = polymerase chain reaction; RT = reverse transcription.

endometriosis [40]. Furthermore, endometriosis-associated ovarian cancer (endometrioid and clear cell carcinoma of the ovary) also displayed a panel of 10 differentially expressed miRNAs [41]. None of these published miRNAs include miR-130a. It remains unclear whether miR-130a can be used as another serum marker for endometriosis.

In the literature, hsa-miR-130a has been mainly regarded as an oncomir. Hsa-miR-130a was reported to downregulate Smad4 and suppress the TGF-β1-induced growth inhibition in granulocytic cells [42]. Hsa-miR-130a may activate Wnt/β-catenin signaling and increase drug resistance in hepatocellular carcinoma cells by inhibiting the RUNX3 pathway [43]. By targeting ATG2B and DICER1, hsa-miR-130a may modulate autophagy as cell survival programs in chronic lymphocytic leukemia cells [44]. In ovarian cancer cells, hsa-miR-130a may be associated with the PI3K/Akt/PTEN/mTOR signaling pathways and play a role in cisplatin resistance [45]. On the other hand, hsa-miR-130a may also be a tumor suppressor. For instance, hsa-miR-130a was shown to induce tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) sensitivity in non-small cell lung cancer cells through the c-Jun-mediated

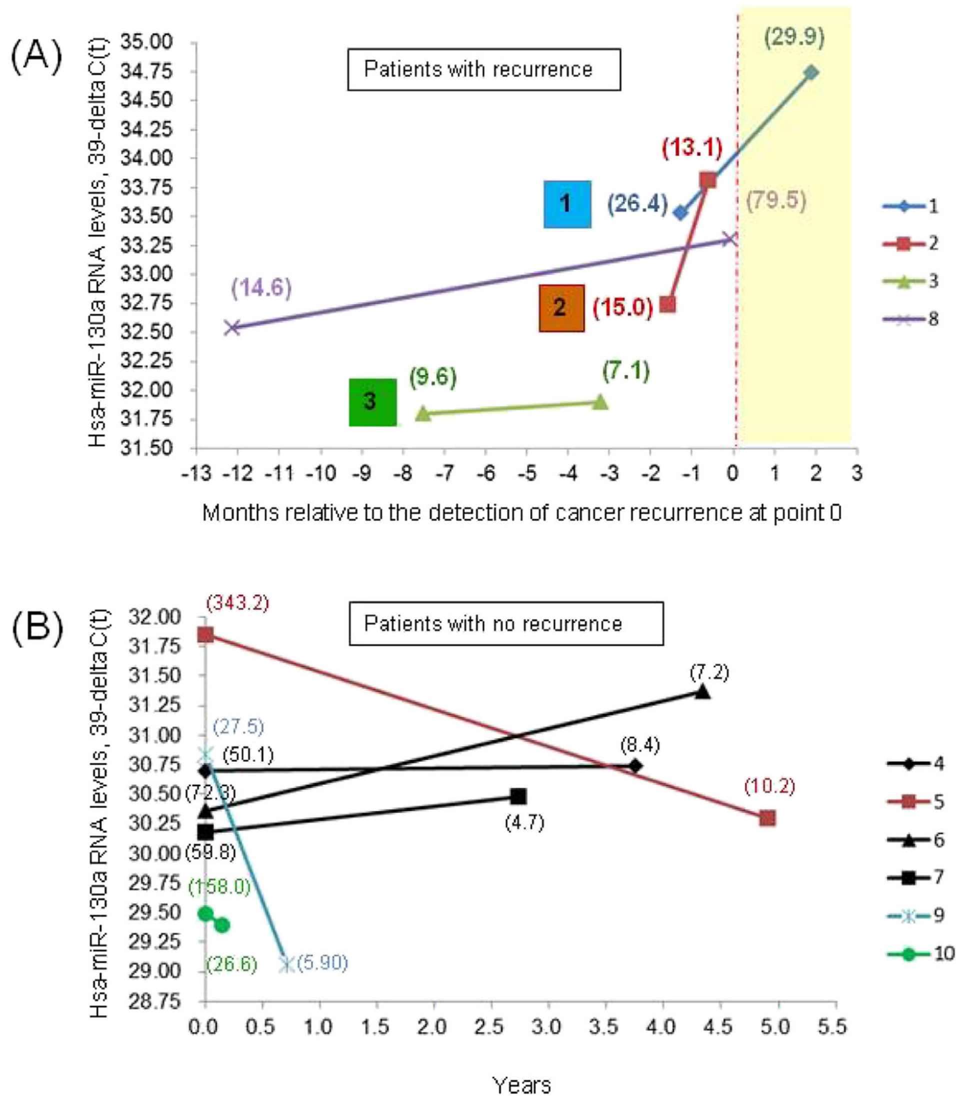


Fig. 3. The changes in hsa-miR-130a in 10 patients with ovarian clear cell cancer. (A) During the follow-up period, four patients had cancer recurrences, which were confirmed by imaging results with computed tomography and/or histological results after operation. (B) During the follow-up period, six patients did not have cancer recurrences after operation. Time zero indicates the date of operation. Serum specimens at the first time point were collected 1 day after operation. The number shown in the parenthesis at each time point is the value of serum CA125 (U/mL).

downregulation of miR-221 and miR-222 [46]. Furthermore, serum miR-130a has been recently used as a biomarker for multiple myeloma [47] and metabolic syndrome [48].

Correlations of miRNAs between tissue and serum are sometimes inconsistent [23,41]. Serum levels of miR-200c could predict the response to chemotherapy and the prognosis of patients with esophageal cancer who received neoadjuvant chemotherapy, but the tissue levels of hsa-miR-200c in these patients did not correlate with the serum level [23]. The results of this study indicated that hsa-miR-187 was highly expressed in preoperative sera (Fig. 2), in agreement with our previous report that hsa-miR-187 was higher in ovarian cancer tissues than in benign tumors [15]. However, hsa-miR-187 failed to serve as a predictor of early recurrence according to the stringent criteria of this study. The mechanism of how cancer-expressed miRNAs enter the circulation warrants further investigation.

Microarray analyses of gene expression have been widely used in cancer tissues [49,50]. miRNA arrays have been used for the purpose of screening candidate miRNAs in ovarian tumors and other cancers [18,19,23,29,32]. In addition, RT-qPCR assays using Taqman [22,24,30,31] or SYBR [26,33] probes were alternative platforms. SYBR RT-qPCR/TaqMan assays protocols have been applied in our previous studies [20,21] and the present investigation. Selection of miRNA reference controls for normalization is required to avoid system bias in such experiments. *C. elegans* miRNAs including cel-miR-238 [23,32], as in this study, and cel-miR-43, cel-miR-54, and cel-miR-238 are often adopted as spike-in controls to reduce technical variation in the experiments [19]. However, this strategy does not exclude the intrinsic biological variations among different study participants [19]. These limitations were also seen in the commonly used miR-16 [19,22,33] and RNU6B [23,24] as endogenous controls. Therefore, inconsistencies may still arise from the use of reference controls even within the same tumor type.

miRNAs are resistant to endogenous RNase activity and show high stability in plasma or serum [16], enabling miRNAs to hold great promises as potential biomarkers. However, there are two problems when utilizing serum or plasma in detecting miRNA levels. First, blood cells contribute partly to the expression of miRNAs within clinical contexts [51,52]. Pritchard et al [51] analyzed 79 circulating miRNA biomarkers for solid tumors and found that myeloid (e.g., hsa-miR-223, hsa-miR-197, hsa-miR-574-3p, and hsa-let-7a) and lymphoid (e.g., hsa-miR-150) blood cells were tightly correlated with corresponding white blood cell counts [51]. Thus, hemolysis can interfere with plasma miRNA levels by up to 50-fold. We excluded hemolysis-affecting miRNAs from our 18-miRNA panel (unpublished data) and avoided hemolyzed specimens that could be identified by the naked eye. However, an objective measure to determine the acceptable level of hemolysis while assessing the circulating miRNAs is lacking. Currently, our understanding of blood components' contribution to the levels of circulating miRNAs may remain incomplete.

Second, the preanalytical and analytical sources of variation in the measurement of circulating miRNAs levels are also likely to have an impact on the results [19,51,52]. If plasma specimens are to be analyzed, sample collection using anticoagulants either with EDTA or heparin should remain consistent throughout the study. Robust methods of RNA extraction and data normalization are critical in the reproducibility and reliability of circulating miRNA assays.

In conclusion, validation of miRNAs as candidate biomarkers is possible if the sources of variation that influence plasma or serum miRNA levels are minimized, and if an appropriate reference of normalization can be identified. A larger sample size is necessary to confirm hsa-miR-130a for its applicability as a potential biomarker in early detection, treatment monitoring, and predicting patient outcome in ovarian cancer.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

Acknowledgments

This study was supported by Ministry of Health and Warfare, Taiwan, Grants DOH102-TD-C-111-006 (to AC and THW), CMR and CLR were sponsored by Chang Gung Memorial Hospital (CMRPG391453/4, 101-2314-B-182-058-MY3 to AC), and (CMRPG391463/4, CLRPG380021, CLRPG380022 to THW). We thanked Jung-Erh Yang and Ying-Yu Lin for excellent technical assistance, and Dr. Shihyee Mimi Wang (Department of Obstetrics and Gynecology, White Memorial Hospital, Los Angeles) for English editing.

References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011;61:69–90.
[2] Prat J. Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchows Arch 2012;460:237–49.
[3] Lim D, Oliva E. Precursors and pathogenesis of ovarian carcinoma. Pathology 2013;45:229–42.
[4] Penson RT, Dizon DS, Birrer MJ. Clear cell cancer of the ovary. Curr Opin Oncol 2013;25:553–7.
[5] Cancer Registry Annual Report, 2008 Taiwan. Department of Health, Executive Yuan; 2010. http://tcr.cph.ntu.edu.tw/main.php?Page=N1.
[6] Ushijima K. Current status of gynecologic cancer in Japan. J Gynecol Oncol 2009;20:67–71.
[7] del Carmen MG, Birrer M, Schorge JO. Clear cell carcinoma of the ovary: a review of the literature. Gynecol Oncol 2012;126:481–90.
[8] Wiegand KC, Shah SP, Al-Agha OM, Zhao Y, Tse K, Zeng T, et al. ARID1A mutations in endometriosis-associated ovarian carcinomas. N Engl J Med 2010;363:1532–43.
[9] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[10] Esquela-Kerscher A, Slack FJ. Oncomirs — microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259–69.
[11] Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proc Natl Acad Sci U S A 2004;101:11755–60.
[12] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834–8.
[13] Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, et al. MicroRNA signatures in human ovarian cancer. Cancer Res 2007;67:8699–707.
[14] Yang H, Kong W, He L, Zhao JJ, O'Donnell JD, Wang J, et al. MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 2008;68:425–33.
[15] Chao A, Lin CY, Lee YS, Tsai CL, Wei PC, Hsueh S, et al. Regulation of ovarian cancer progression by microRNA-187 through targeting Disabled homolog-2. Oncogene 2012;31:764–75.
[16] Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513–8.
[17] Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. Cell Res 2008;18:997–1006.
[18] Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One 2009;4:e6229.
[19] Huang X, Liang M, Dittmar R, Wang L. Extracellular microRNAs in urologic malignancies: chances and challenges. Int J Mol Sci 2013;14:14785–99.
[20] Chen HC, Chen GH, Chen YH, Liao WL, Liu CY, Chang KP, et al. MicroRNA deregulation and pathway alterations in nasopharyngeal carcinoma. Br J Cancer 2009;100:1002–11.
[21] Huang Y, Chen HC, Chiang CW, Yeh CT, Chen SJ, Chou CK. Identification of a two-layer regulatory network of proliferation-related microRNAs in hepatoma cells. Nucleic Acids Res 2012;40:10478–93.
[22] Koberle V, Kronenberger B, Pleli T, Trojan J, Imelmann E, Peveling-Oberhag J, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer 2013;49:3442–9.
[23] Tanaka K, Miyata H, Yamasaki M, Sugimura K, Takahashi T, Kurokawa Y, et al. Circulating miR-200c levels significantly predict response to chemotherapy

and prognosis of patients undergoing neoadjuvant chemotherapy for esophageal cancer. Ann Surg Oncol 2013;20(Suppl. 3):S607–15.

[24] Yang IP, Tsai HL, Huang CW, Huang MY, Hou MF, Juo SH, et al. The functional significance of microRNA-29c in patients with colorectal cancer: a potential circulating biomarker for predicting early relapse. PLoS One 2013;8:e66842.

[25] Heegaard NH, Schetter AJ, Welsh JA, Yoneda M, Bowman ED, Harris CC. Circulating micro-RNA expression profiles in early stage nonsmall cell lung cancer. Int J Cancer 2012;130:1378–86.

[26] Asaga S, Kuo C, Nguyen T, Terpenning M, Giuliano AE, Hoon DS. Direct serum assay for microRNA-21 concentrations in early and advanced breast cancer. Clin Chem 2011;57:84–91.

[27] Morimura R, Komatsu S, Ichikawa D, Takeshita H, Tsujiura M, Nagata H, et al. Novel diagnostic value of circulating miR-18a in plasma of patients with pancreatic cancer. Br J Cancer 2011;105:1733–40.

[28] Tsujiura M, Ichikawa D, Komatsu S, Shiozaki A, Takeshita H, Kosuga T, et al. Circulating microRNAs in plasma of patients with gastric cancers. Br J Cancer 2010;102:1174–9.

[29] Taylor DD, Gercel-Taylor C. MicroRNA signatures of tumor-derived exosomes as diagnostic biomarkers of ovarian cancer. Gynecol Oncol 2008;110:13–21.

[30] Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol 2009;112:55–9.

[31] Kan CW, Hahn MA, Gard GB, Maidens J, Huh JY, Marsh DJ, et al. Elevated levels of circulating microRNA-200 family members correlate with serous epithelial ovarian cancer. BMC Cancer 2012;12:627.

[32] Chung YW, Bae HS, Song JY, Lee JK, Lee NW, Kim T, et al. Detection of microRNA as novel biomarkers of epithelial ovarian cancer from the serum of ovarian cancer patient. Int J Gynecol Cancer 2013;23:673–9.

[33] Guo F, Tian J, Lin Y, Jin Y, Wang L, Cui M. Serum microRNA-92 expression in patients with ovarian epithelial carcinoma. J Int Med Res 2013;41(5):1456–61.

[34] Duffy MJ, Bonfrer JM, Kulpa J, Rustin GJ, Soletormos G, Torre GC, et al. CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. Int J Gynecol Cancer 2005;15:679–91.

[35] Jacobs I, Bast Jr RC. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod 1989;4:1–12.

[36] Chao A, Lee LY, Hsueh C, Lin CY, Tsai CL, Chao AS, et al. Immunohistological analysis of stress-induced phosphoprotein 1 in ovarian cancer patients with low serum cancer antigen 125 levels. Taiwan J Obstet Gynecol 2013;52:185–91.

[37] Ness RB. Endometriosis and ovarian cancer: thoughts on shared pathophysiology. Am J Obstet Gynecol 2003;189:280–94.

[38] Pearce CL, Templeman C, Rossing MA, Lee A, Near AM, Webb PM, et al. Association between endometriosis and risk of histological subtypes of ovarian cancer: a pooled analysis of case-control studies. Lancet Oncol 2012;13:385–94.

[39] Jia SZ, Yang Y, Lang J, Sun P, Leng J. Plasma miR-17-5p, miR-20a and miR-22 are down-regulated in women with endometriosis. Hum Reprod 2013;28:322–30.

[40] Wang WT, Zhao YN, Han BW, Hong SJ, Chen YQ. Circulating microRNAs identified in a genome-wide serum microRNA expression analysis as noninvasive biomarkers for endometriosis. J Clin Endocrinol Metab 2013;98:281–9.

[41] Suryawanshi S, Vlad AM, Lin HM, Mantia-Smaldone G, Laskey R, Lee M, et al. Plasma microRNAs as novel biomarkers for endometriosis and endometriosis-associated ovarian cancer. Clin Cancer Res 2013;19:1213–24.

[42] Hager M, Pedersen CC, Larsen MT, Andersen MK, Hother C, Gronbaek K, et al. MicroRNA-130a-mediated down-regulation of Smad4 contributes to reduced sensitivity to TGF-beta1 stimulation in granulocytic precursors. Blood 2011;118:6649–59.

[43] Xu N, Shen C, Luo Y, Xia L, Xue F, Xia Q, et al. Upregulated miR-130a increases drug resistance by regulating RUNX3 and Wnt signaling in cisplatin-treated HCC cell. Biochem Biophys Res Commun 2012;425:468–72.

[44] Kovaleva V, Mora R, Park YJ, Plass C, Chiramel AI, Bartenschlager R, et al. miRNA-130a targets ATG2B and DICER1 to inhibit autophagy and trigger killing of chronic lymphocytic leukemia cells. Cancer Res 2012;72:1763–72.

[45] Yang L, Li N, Wang H, Jia X, Wang X, Luo J. Altered microRNA expression in cisplatin-resistant ovarian cancer cells and upregulation of miR-130a associated with MDR1/P-glycoprotein-mediated drug resistance. Oncol Rep 2012;28:592–600.

[46] Acunzo M, Visone R, Romano G, Veronese A, Lovat F, Palmieri D, et al. miR-130a targets MET and induces TRAIL-sensitivity in NSCLC by downregulating miR-221 and 222. Oncogene 2012;31:634–42.

[47] Kubiczkova L, Kryukov F, Slaby O, Dementyeva E, Jarkovsky J, Nekvindova J, et al. Circulating serum microRNAs as novel diagnostic and prognostic biomarkers for multiple myeloma and monoclonal gammopathy of undetermined significance. Haematologica 2014;99:511–8.

[48] Zhou J, Zheng Q, Xu T, Liao D, Zhang Y, Yang S, et al. Associations between physical activity-related miRNAs and metabolic syndrome. Horm Metab Res 2014;46:201–5.

[49] Wang TH, Chao A. Microarray analysis of gene expression of cancer to guide the use of chemotherapeutics. Taiwan J Obstet Gynecol 2007;46:222–9.

[50] Chao A, Wang TH, Lai CH. Overview of microarray analysis of gene expression and its applications to cervical cancer investigation. Taiwan J Obstet Gynecol 2007;46:363–73.

[51] Pritchard CC, Kroh E, Wood B, Arroyo JD, Dougherty KJ, Miyaji MM, et al. Blood cell origin of circulating microRNAs: a cautionary note for cancer biomarker studies. Cancer Prev Res (Phila) 2012;5:492–7.

[52] Duttagupta R, Jiang R, Gollub J, Getts RC, Jones KW. Impact of cellular miRNAs on circulating miRNA biomarker signatures. PLoS One 2011;6:e20769.