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# Serum exosomal microRNA-122 and microRNA-21 as predictive biomarkers in transarterial chemoembolization-treated hepatocellular carcinoma patients

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**Abstract.** Exosomal microRNAs (miRNAs) have been investigated as potential novel biomarkers, and miR-122 and miR-21 were shown to be important in hepatocellular carcinoma (HCC). We analyzed the importance of serum exosomal miRNA expression levels in HCC patients that underwent transarterial chemoembolization (TACE). Seventy-five HCC patients who underwent TACE as the initial treatment in Nagasaki University Hospital were enrolled. Exosomal miRNAs were isolated from serum samples collected before and after TACE. Exosomal miR-122 expression levels significantly decreased after TACE (P=0.012), while the exosomal miR-21 expression levels did not significantly change. The expression levels of exosomal miR-122 before TACE were shown to correlate significantly with aspartate aminotransferase (r=0.31, P=0.004) and alanine aminotransferase (r=0.33, P=0.003) levels, tumor diameter (r=0.29, P=0.010) and Child-Pugh score (r=-0.28, P=0.013). The median survival time for all patients was 47 months, and neither of the investigated exosomal miRNAs were shown to be independent factors associated with the disease-specific survival. According to the median relative expression of miR-122 after TACE/before TACE (miR-122 ratio) in liver cirrhosis patients (n=57), the patients with a higher miR-122 ratio had significantly longer disease-specific survival, compared with that of the patients with the lower miR-122 ratio (P=0.0461). Multivariate Cox proportional hazards regression analysis of clinical parameters revealed that a lower exosomal miR-122 ratio (HR 2.720; 95% confidence interval, 1.035-8.022; P=0.042) is associated with the disease-specific survival. Taken together, our results demonstrate that the exosomal miR-122 level alterations may represent a predictive biomarker in HCC patients with liver cirrhosis treated with TACE.

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*Key words:* carcinoma, hepatocellular, chemoembolization, therapeutic, exosome, microRNA

## Introduction

Hepatocellular carcinoma (HCC) develops from chronic liver diseases and it represents a common cancer type worldwide, with high incidence in East and South Asia (1,2). Various HCC treatments exist, such as resection, radiofrequency ablation, irradiation, and chemotherapy, but transarterial chemoembolization (TACE) is usually performed (3,4), as this selective intervention may result in a favorable clinical course (5). However, the prognosis of multiple HCCs remains poor due to a high recurrence rate and resistance to chemotherapy (6). In order to improve the disease prognosis and evaluate the effectiveness of TACE, the identification of non-invasive predictive biomarkers is required.

MicroRNAs (miRNAs) are small non-coding RNAs (17-23 nucleotides) that regulate mRNA post-transcriptionally, and many miRNAs have been reported to be potential predictive biomarkers (7). miR-122 is one of the miRNAs highly expressed in liver (8), and a decrease in its expression in HCC patients was shown to be associated with hepatocarcinogenesis and poor prognosis (9). Additionally, cyclin G1, a disintegrin and metalloprotease 17 (ADAM17), and IGF1R are the targets of miR-122 (10), shown to be downregulated in HCC tissues (11). In contrast, miR-21 was shown to be overexpressed in some malignancies, and it plays an important role in cell proliferation, invasion, and migration, by suppressing PTEN expression (12).

Exosomes are extracellular vesicles, 40-100 nm large, which can contain different molecules, including proteins, DNA, RNA, and miRNAs, but their content does not necessarily mirror the RNA expression profile and can change in response to cellular conditions (13). Although the exosomal miRNAs found in patient sera were shown to be associated with the clinical features in different malignances (14,15), TACE-induced changes in the exosomal miRNA expression levels remain unknown. Therefore, we hypothesized that the exosomal miR-122 and miR-21 may play a key role in HCC

development and progression and investigated whether the exosomal miRNA ratio can be used as a predictive marker in HCC patients treated with TACE.

## Materials and methods

*Patients and samples*. Seventy-five HCC patients who underwent TACE as the initial treatment at Nagasaki University Hospital (Nagasaki, Japan) from January 2006 to March 2013 were enrolled in this study. This study was approved by the Research Ethics Committee of Nagasaki University Hospital, and we obtained informed consent from all patients. Liver function before the treatment was preserved in all patients, and patients with Child-Pugh grade C or with the portal vein obstruction due to tumor thrombosis were excluded from the study. We used the Barcelona Clinic Liver Cancer staging classification (BCLC) for clinical staging. The average age at the treatment was 73 (range, 38-89) and the number of patients with liver cirrhosis (LC) was 57 (76%) and with chronic hepatitis, 18 (24%) (Table I). HCC patients were diagnosed by using contrast-enhanced-computed tomography (CE-CT) or Gd-EOB-DTPA-enhanced magnetic resonance imaging (MRI), which revealed the nodules with early enhancement in arterial phase and washout in portal or venous phase. Serum samples were obtained before TACE and approximately 7 days after the treatment, and they were preserved at -80˚C until exosome extraction.

*TACE treatment*. The conventional TACE was performed using a mixture of epirubicin hydrochloride or miriplatin hydrate, mitomycin C, iodine addition products of the fatty acid ethyl esters obtained from the poppy-seed oil, and a contrast agent, administered into the tumor vessels. Subsequently, embolization was performed using gelatin sponge particles if a massive tumor thrombosis did not appear following the treatment.

*Exosome isolation from human serum samples*. ExoQuick (63 $\mu$l; System Biosciences, Palo Alto, CA, USA) was added to 250 $\mu$l of cell-free serum samples, and the mixture was placed at 4˚C overnight. The mixture was centrifuged at 1,500 x g for 30 min and the supernatant was removed to obtain the exosome pellets. The pellets were resuspended in 300 $\mu$l of the lysis buffer for RNA isolation and 5 $\mu$l of *Caenorhabditis elegans* microRNA (*Cel-miR-39,* mirVana miRNA mimic; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added to normalize the levels.

Table I. Clinical characteristics of 75 patients pre-TACE.

| Characteristic | Median (range) or number (%) |
|---|---|
| Age (years) | 73 (38-89) |
| Sex (%) | |
| Male | 49 (65) |
| Female | 26 (35) |
| Liver cirrhosis (%) | 57 (76) |
| Chronic hepatitis (%) | 18 (24) |
| Etiology (%) | |
| HBV | 9 (12) |
| HBV+HCV | 3 (4) |
| HCV | 39 (52) |
| NBNC | 24 (32) |
| BCLC stage (%) | |
| 0 | 1 (1) |
| A | 36 (48) |
| B | 34 (46) |
| C | 4 (5) |
| Type 2 diabetes (%) | |
| Positive | 30 (40) |
| Negative | 45 (60) |
| AST (IU/l) | 47 (13-219) |
| ALT (IU/l) | 33 (9-220) |
| HbA1c (%) | 5.5 (4.1-8.4) |
| T-Bil (mg/dl) | 0.9 (0.3-4.2) |
| ALB (g/dl) | 3.5 (2.3-5.0) |
| PT (%) | 75 (47-118) |
| Plt (x$10^4$/l) | 11.1 (2.6-39.7) |
| Child-Pugh score | 6 (5-9) |
| AFP (pg/ml) | 15.8 (1-126234) |
| DCP (AU/ml) | 96 (7-225827) |
| Tumor diameter (cm) | 3.4 (1-19.5) |
| Tumor number | 2 (1-10) |

AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycohemoglobin A1c; T-Bil, total bilirubin; ALB, albumin; PT, prothrombin time; Plt, platelet; AFP, α-fetoprotein; DCP, des-γ carboxyprothrombin, BCLC, Barcelona Clinic Liver Cancer staging classification; TACE, transarterial chemoembolization.

*RNA isolation and RT-qPCR assay*. MirVana miRNA Isolation Kit (Invitrogen; Thermo Fisher Scientific, Inc.) was used to isolate RNA. TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for the reverse transcription of miRNA to cDNA, using miR-122, miR-21 and cel-miR-39 specific primers (TaqMan MicroRNA Assays; Applied Biosystems; Thermo Fisher Scientific, Waltham, Inc.).

After the synthesis of cDNA, RT-qPCR was performed by using TaqMan Universal Mastermix II (Applied Biosystems; Thermo Fisher Scientific, Inc.) and RT-qPCR reactions were performed using LightCycler 480 system II (Roche Diagnostics, Basel, Switzerland), and Cq values were calculated. The assays were performed in triplicate, and the relative quantification of miRNA expression levels was performed using the $2^{-\Delta\Delta Cq}$ method ($\Delta Cq=Cq_{miR}-Cq_{cel\text{-}miR\text{-}39}$) (16). Because of the non-normal distribution, the logarithmic transformation of the relative expression levels of exosomal miRNAs was used for analyses. Exosomal miRNA ratio was defined as the fold change of ΔCq (miR ratio=ΔCq miR after TACE/ΔCq miR before TACE).

*Statistical analysis*. Clinicopathological data are presented as median (range). JMP Pro 11.2.0 (SAS Institute Inc., Cary, NC, USA) was used for the statistical analysis. The Student's t-test was used to analyze paired data, while the correlations were

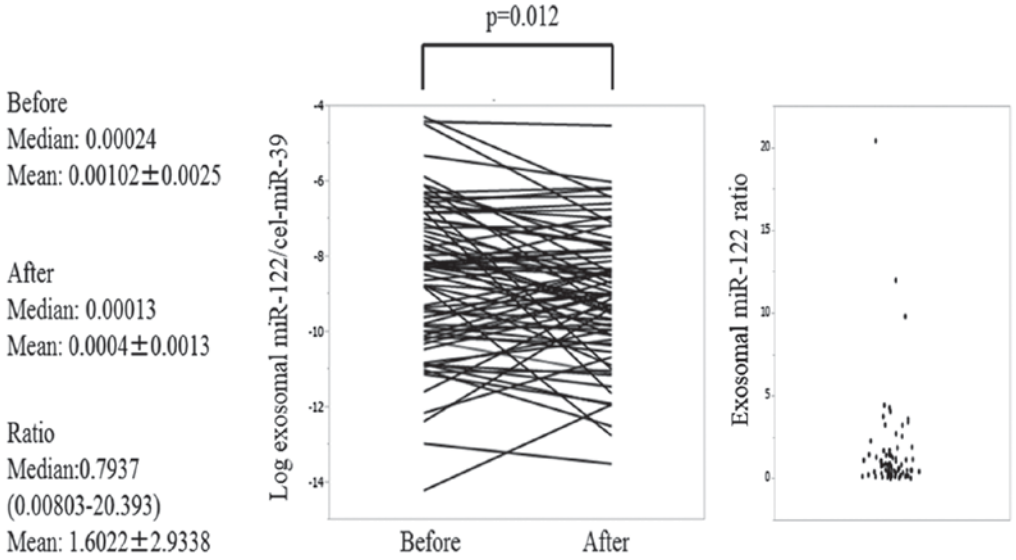


Figure 1. Exosomal miR-122 expression level changes after TACE. Exosomal miR-122 expression levels significantly decreased after TACE, compared with the control (P=0.012). miR, microRNA; TACE, transarterial chemoembolization.

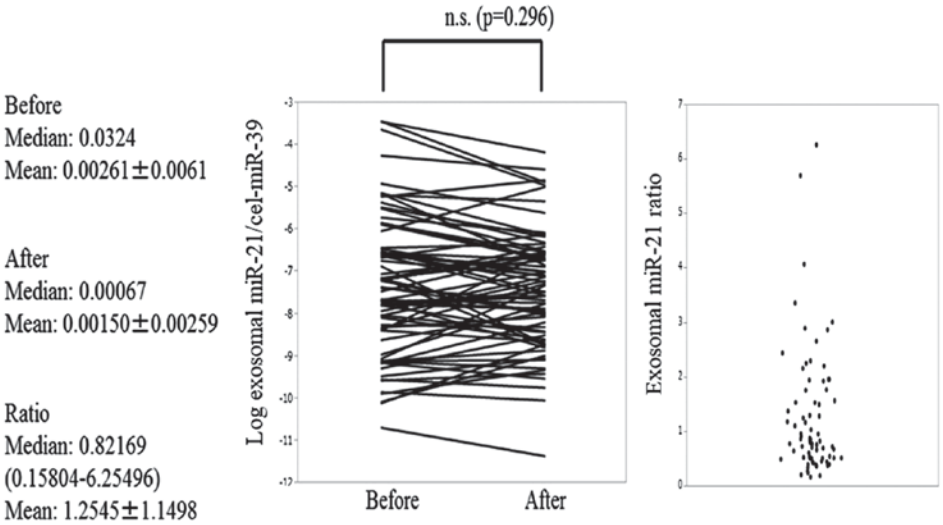


Figure 2. Exosomal miR-21 expression level changes after TACE. Exosomal miR-21 expression levels did not differ pre- and post-TACE (P=0.296). miR, microRNA; TACE, transarterial chemoembolization.

analyzed using the Pearson's correlation coefficient. The cumulative survival rate was calculated using Kaplan-Meier method and the difference in the survival time between two groups was assessed with log-rank test. Univariate and multivariate Cox proportional hazard analyses were used to calculate Cox proportional hazard ratio between disease specific survival and clinical parameter. P-values were bilaterally tested, and P<0.05 was considered to indicate a statistically significant difference.

## Results

*TACE-induced exosomal miRNA alterations and the correlations between the pre-TACE exosomal miRNA levels and clinical parameters*. Exosomal miR-122 expression levels were shown to be significantly decreased after TACE (P=0.012) (Fig. 1), but the exosomal miR-21 expression levels did not change (Fig. 2). The expression levels of exosomal miR-122 before TACE were shown to be significantly correlated with aspartate aminotransferase (AST) levels (r=0.31, P=0.004), alanine aminotransferase (ALT) levels (r=0.33, P=0.003), tumor diameter (r=0.29, P=0.010), and Child-Pugh score (r=-0.28, P=0.013; Table II). Exosomal miR-21 pre-TACE levels were shown to correlate with prothrombin time (r=0.38, P<0.001) and Child-Pugh score (r=-0.30, P=0.006; Table III).

Exosomal miR-122 and miR-21 pre-TACE expression levels did not significantly differ between the LC and chronic hepatitis groups. However, exosomal miR-122 pre-TACE expression levels were significantly higher in the Child-Pugh grade A patients than in the Child-Pugh grade B patients (ANOVA, P=0.0090). Similar results were obtained when miR-21 expression was analyzed (ANOVA, P=0.0022).

*Association between clinical parameters and exosomal miRNA levels and disease-specific survival*. The MST for

Table II. Correlation between pre-TACE exosomal miR-122 levels and clinical parameters.

| Variable | r | Case number | Lower 95% CI | Upper 95% CI | P-value |
|---|---|---|---|---|---|
| Age (years) | -0.1594 | 75 | -0.3729 | 0.0701 | 0.172 |
| HbA1c (%) | 0.1528 | 70 | -0.0852 | 0.3743 | 0.206 |
| AST (IU/l) | 0.3144 | 75 | 0.0942 | 0.5053 | 0.006 |
| ALT (IU/l) | 0.3365 | 75 | 0.1186 | 0.5235 | 0.003 |
| T-Bil (mg/dl) | -0.1892 | 75 | -0.399 | 0.0395 | 0.104 |
| ALB (g/dl) | 0.1092 | 75 | -0.1208 | 0.328 | 0.351 |
| PT (%) | 0.2209 | 73 | -0.0097 | 0.4291 | 0.060 |
| Plt ($x10^4$/l) | 0.1423 | 75 | -0.0874 | 0.3578 | 0.223 |
| Child Pugh score | -0.2838 | 75 | -0.4798 | -0.0607 | 0.013 |
| AFP (pg/ml) | 0.1893 | 75 | -0.0394 | 0.3991 | 0.103 |
| DCP (AU/ml) | 0.2194 | 73 | -0.0112 | 0.4279 | 0.062 |
| Tumor diameter (cm) | 0.2933 | 75 | 0.0711 | 0.4878 | 0.010 |
| Tumor number | 0.1461 | 75 | -0.0837 | 0.3611 | 0.211 |

r, Pearson's correlation coefficient; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycohemoglobin A1c; T-Bil, total bilirubin; ALB, albumin; CI, confidence interval; PT, prothrombin time; Plt, platelet; AFP, α-fetoprotein; DCP, des-γ carboxyprothrombin; TACE, transarterial chemoembolization.

Table III. Correlation between pre-TACE exosomal miR-21 levels and clinical parameters.

| Variable | r | Case number | Lower 95% CI | Upper 95% CI | P-value |
|---|---|---|---|---|---|
| Age (years) | 0.0487 | 75 | -0.1802 | 0.2727 | 0.678 |
| HbA1c (%) | 0.1242 | 70 | -0.1141 | 0.349 | 0.305 |
| AST (IU/l) | 0.1937 | 75 | -0.0348 | 0.403 | 0.095 |
| ALT (IU/l) | 0.2248 | 75 | -0.0023 | 0.4298 | 0.052 |
| T-Bil (mg/dl) | -0.1781 | 75 | -0.3893 | 0.051 | 0.126 |
| ALB (g/dl) | 0.1932 | 75 | -0.0353 | 0.4025 | 0.096 |
| PT (%) | 0.3846 | 73 | 0.1696 | 0.5647 | <0.001 |
| Plt ($x10^4$/l) | 0.1831 | 75 | -0.0457 | 0.3937 | 0.115 |
| Child Pugh score | -0.3093 | 75 | -0.5011 | -0.0886 | 0.006 |
| AFP (pg/ml) | 0.1495 | 75 | -0.0802 | 0.3641 | 0.200 |
| DCP (AU/ml) | 0.1168 | 73 | -0.1164 | 0.3378 | 0.325 |
| Tumor diameter (cm) | 0.1263 | 75 | -0.1036 | 0.3434 | 0.280 |
| Tumor number | -0.0639 | 75 | -0.2867 | 0.1655 | 0.586 |

r, Pearson's correlation coefficient; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycohemoglobin A1c; T-Bil, total bilirubin; ALB, albumin; CI, confidence interval; PT, prothrombin time; Plt, platelet; AFP, α-fetoprotein; DCP, des-γ carboxyprothrombin; TACE, transarterial chemoembolization.

all patients was 47 months. Univariate analysis of factors associated with survival demonstrated that Child-Pugh grade B (hazard ratio (HR), 2.495; 95% confidence interval (CI), 1.186-5.218; P=0.016), α-fetoprotein (AFP) levels >20 pg/ml (HR, 2.186; 95% CI, 1.061-4.609; P=0.034), des-gammma-carboxyprothrombin (DCP) levels >40 AU/ml (HR, 3.240, 95% CI, 1.248-11.06; P=0.013), and tumor diameter >3 cm (HR, 2.426; 95% CI, 1.132-5.637; P=0.022) represent the risk factors. Multivariate Cox proportional hazard regression results showed that Child-Pugh grade B (HR, 5.172; 95% CI, 2.194-12.586; P=0.0002), AFP >20 pg/ml (HR, 2.667; 95% CI, 1.197-6.147; P=0.0163), DCP >40 AU/ml (HR, 4.695; 95% CI, 1.674-17.051), and tumor diameter >3 cm (HR, 2.844; 95% CI, 1.266-6.931; P=0.0106) represent independent risk factors. However, exosomal miR-122 and miR-21 expression levels, exosomal miR-122 ratio, and exosomal miR-21 ratio were not shown to be associated with the disease-specific survival.

In the limited LC group (n=57), according to median exosomal miR-122 ratio, the patients with the higher ratio showed a significantly longer disease-specific survival than that observed in the group with the lower ratio (P=0.0461)

Table IV. Factors associated with the disease-specific survival in liver cirrhosis patients (n=57).

| Variable | HR (95% CI) | P-value | HR (95% CI) | P-value |
|---|---|---|---|---|
| Sex (male vs. female) | 0.545 (0.236-1.320) | 0.172 | - | - |
| Age (years >60 vs. ≦60) | 0.678 (0.300-1.623) | 0.369 | - | - |
| Type 2 diabetes (positive vs. negative) | 0.433 (0.155-1.044) | 0.063 | - | - |
| HBV (positive vs. negative) | 2.144 (0.610-5.896) | 0.209 | - | - |
| AST (>50 vs. ≦50 U/l) | 1.158 (0.489-2.664) | 0.731 | - | - |
| ALT (>50 vs. ≦50 U/l) | 0.838 (0.301-2.029) | 0.708 | - | - |
| Child-Pugh grade (B vs. A) | 2.372 (1.011-5.685) | 0.047 | 3.588 (1.446-9.224) | 0.006 |
| AFP (>20 vs. ≦20 pg/ml) | 2.225 (0.968-5.378) | 0.059 | | |
| DCP (>40 vs ≦40 AU/ml) | 2.810 (1.046-9.763) | 0.039 | 2.960 (0.984-11.238) | 0.053 |
| Tumor diameter (>3 vs. ≦3 cm) | 2.554 (1.101-6.387) | 0.028 | 3.606 (1.484-9.505) | 0.004 |
| Tumor number (≧2 vs. 1) | 1.007 (0.412-2.816) | 0.987 | - | - |
| BCLC (B+C vs 0+A) | 1.108 (0.492-2.585) | 0.804 | - | - |
| Exo miR122 (≦0.00024 vs. >0.00024) | 0.754 (0.329-1.743) | 0.502 | - | - |
| Exo miR122 ratio (≦0.793 vs. >0.793) | 2.490 (1.028-6.689) | 0.042 | 2.720 (1.035-8.022) | 0.042 |
| Exo miR21 (≦0.00064 vs. >0.00064) | 1.049 (0.466-2.407) | 0.906 | - | - |
| Exo miR21 ratio (≦0.82169 vs. >0.82169) | 1.293 (0.564-2.996) | 0.539 | - | - |

Cox proportional hazards regression was performed to derive HR and CI values. HBV, hepatitis B virus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HbA1c, glycohemoglobin A1c; T-Bil, total bilirubin; ALB, albumin; PT, prothrombin time; Plt, platelet; AFP, α-fetoprotein; DCP, des-γ carboxyprothrombin; BCLC, Barcelona Clinic Liver Cancer staging; TACE, transarterial chemoembolization.

(Fig. 3). No significant difference in disease-specific survival was observed between the patients with the higher and lower exosomal miR-21 ratios. Univariate Cox proportional hazards regression revealed that Child-Pugh grade B (HR, 2.372; 95% CI, 1.011-5.685; P=0.047), DCP >40 AU/ml (HR, 2.810; 95% CI, 1.046-9.763; P=0.0396), tumor diameter >3 cm (HR, 2.554; 95% CI, 1.101-6.387; P=0.0287), and the decrease in the exosomal miR-122 ratio (HR, 2.490; 95% CI, 1.028-6.689; P=0.0429) represent risk factors (Table IV). Multivariate Cox proportional hazards regression results demonstrated here that the Child-Pugh grade B (HR, 3.588; 95% CI, 1.446-9.224; P=0.006), tumor diameter >3 cm (HR, 3.606; 95% CI, 1.484-9.505; P=0.004), and lower exosomal miR-122 ratio (HR, 2.720; 95% CI, 1.035-8.022; P=0.042) represent independent factors associated with poor prognosis.

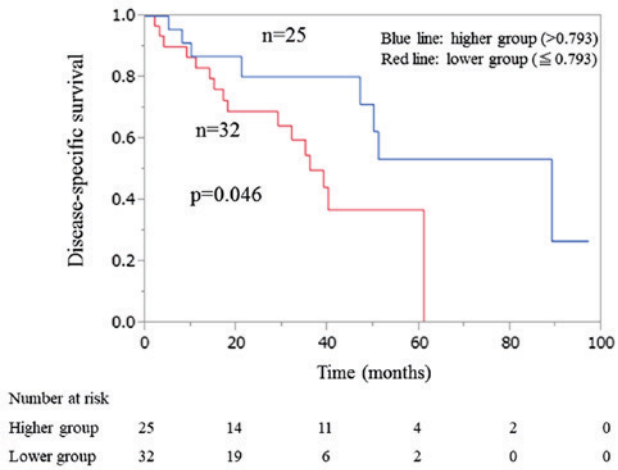


Figure 3. Cumulative survival of LC patients according to the exosomal miR-122 ratio. Kaplan-Meier survival analysis of disease-specific survival according to the miR-122 ratio in LC patients. Patients with a higher miR-122 ratio were shown to have a significantly longer survival time compared with that in the other group (P=0.046). miR, microRNA; LC, liver cirrhosis.

## Discussion

Treatment strategy for HCC patients requires obtaining the accurate data showing the tumor stage and residual liver function, due to the potential post-treatment liver failure. Therefore, biomarkers that may provide liver-specific information are required. Several studies analyzed the pre-treatment expression levels of exosomal miRNAs in HCC patients (17,18), however, there are no reports describing the alterations in miRNA levels after TACE. To the best of our knowledge, this is the first report showing that the exosomal miRNA ratio affects the prognosis of LC patients.

Liver function usually deteriorates after TACE, due to the damaging of the non-cancerous tissue. We showed that the post-TACE exosomal miR-122 levels significantly decreased, and no correlation between exosomal miRNA levels and conventional tumor marker levels was observed.The expression levels of miR-122 after TACE significantly inversely correlated with the Child-Pugh score (r=-0.24, P=0.0344), however, these changes did not correlate with the changes in the standard liver function tests. These results suggest that the decline in exosomal miR-122 levels may reflect a decrease in the liver function, rather than the anti-tumor effects of this procedure. In contrast, exosomal miR-21 levels were not significantly altered after TACE. A previous report showed the increased expression of serum exosomal miR-21 in HCC patients with HBV (19)

but no previous studies reported the changes in the exosomal miR-21 level following the TACE treatment.

Recent studies demonstrated that the circulating miR-122 levels are associated with the liver damage and ALT levels (20-23). In our study, the expression levels of serum exosomal miR-122 before TACE were shown to correlate significantly with AST and ALT levels,. Pre-TACE exosomal miR-122 levels were shown to be negatively correlated with the Child-Pugh score, indicating that the expression levels of exosomal miR-122 reflect liver function and liver fibrosis rate. In our previous study, we showed that a decrease in serum miR-122 levels correlates with the development of severe fibrosis in patients with non-alcoholic fatty liver disease (NAFLD) (24), while Morita *et al* (25) showed that the hepatic miR-122 levels in patients with HCV are negatively correlated with the functional liver damage. If exosomal miRNA levels mirror those in the parental cells, the obtained result may indicate that the exosomal miR-122 levels reflect residual liver function and capacity.

Pre-TACE expression levels of exosomal miR-122 did not significantly differ between the chronic hepatitis and LC patient groups. miR-122 was shown to be associated with the liver fibrosis rate (24,26,27) and viral replication rate (28-30) and therefore, the heterogeneous patient background may affect the obtained results. Furthermore, no significant correlation was observed between the expression levels of exosomal miR-21 and the BCLC stage. Exosomal miR-21 levels were shown to be associated with prothrombin time and Child-Pugh score, and therefore, this molecule can represent a less specific prognostic biomarker than exosomal miR-122 in HCC patients.

The mechanisms of action and functions of miR-122, especially post-treatment, are not well-known. In hypoxic condition after embolization, this molecule may induce hypoxia inducible factor 1α (HIF-1α) expression in non-cancerous liver tissue and cancer cells. HIF-1α and vimentin represent miR-122 targets in hepatocytes (31), while the reduced liver miR-122 levels were shown to be associated with increased HIF-1α levels in the diet-induced steatohepatitis mouse model. There is a possibility that the changes in miR-122 levels are associated with epithelial-mesenchymal transition (EMT), however, we were not able to show whether the EMT is associated with decline of miR-122 in this study. A recent study demonstrated that miR-122 inhibits the EMT by targeting Snail and WNT/β-cadherin signaling pathway (32). Additionally, miR-122 plays a key role in mitochondrial metabolism by indirectly regulating mitochondrial genes (33), such as PPARGC1A (PGC-1a). The loss of miR-122 can result in a damaged liver function (33) and it was shown to be associated with the HCC patient mortality.

In all patients, exosomal miR-122 and miR-21 levels, and miRNA ratios were not shown to be independent factors associated with the disease-specific survival. However, in HCC patients with LC, lower exosomal miR-122 ratio was shown to be an independent factor for poor prognosis. A previous report showed that serum miR-122 levels negatively correlate with the model of end-stage liver disease (MELD) score (34) and are associated with poor prognosis in decompensated liver disease patients (35). Moreover, in HCV-induced fibrosis, the decrease in circulating miR-122 reflects the development of liver fibrosis and the loss of viable liver cells (36). Therefore, liver fibrosis contributes to the liver function decline, and our results indicate that exosomal miR-122 levels, especially in LC patients, may serve as important post-TACE predictive biomarkers. Since a considerably higher decline in exosomal miR-122 levels after TACE occurs in patients with LC than in those with chronic hepatitis, we hypothesize that the group with a more prominent decrease in exosomal miR-122 levels after TACE has a lower survival rate.

Several limitations of this study should be noted. To determine tumor-specific exosomal miRNAs, exosomal miRNA levels in the sera of patients without HCC, but with chronic liver diseases, should be determined. Our study was retrospective, with a somewhat small sample size, and it included advanced chronic hepatitis cases, because all patients with preserved liver function underwent the surgical procedure.

In conclusion, exosomal miR-122 levels may reflect the liver damage and residual liver function levels. This is the first report showing that the post-TACE expression levels of exosomal miR-122 decrease, especially in the LC patients. Additionally, lower miR-122 ratio was shown to be associated with poor prognosis. Serum exosomal miRNA levels after treatment may represent novel biomarkers guiding the decision-making during the treatment of HCC patients.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

HM designed the study, developed the methodology and reviewed the final version of the manuscript. TS performed data analyses, curated and visualized data, and wrote the first draft. TS and YK performed the experiments. KN supervised the study. All authors read and approved the final version of the manuscript. Resources: HS, TH, EO, SM, NT and KN. HS, TH, EO, SM, NT and KN contributed to the acquisition and interpretation of data

**Ethics approval and consent to participate**

This study was approved by the Research Ethics Committee of Nagasaki University Hospital (no. 16042513), and informed consent was obtained from all patients.

**Consent for publication**

Written informed consents was obtained from all patients included in the study.

**Competing interests**

The authors declare that they have no competing interests.

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