Preintervention MCP-1 serum levels as an early predictive marker of tumor response in patients with hepatocellular carcinoma undergoing transarterial chemoembolization

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Background: Transarterial chemoembolization (TACE) is a widely accepted treatment for unresectable or intermediate-stage hepatocellular carcinoma (HCC). However, response rates to TACE are heterogeneous and it is not fully understood which patients benefit most from TACE therapy in terms of tumor response. To identify the possible predictive roles of the perioperative monocyte chemoattractant protein-1 (MCP-1) levels in patients of HCC treated with TACE.

Methods: Forty patients of HCC receiving TACE were enrolled in a single center prospective observational study. MCP-1 and miR-210 levels were measured in 40 HCC patients at baseline before TACE and compared with 17 healthy controls by immunoassay and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Tumor response assessments were taken after TACE treatment 4–6 weeks. Univariate and multivariate analysis were conducted to analyze factors correlated with tumor response in a Logistic regression model. The predictive roles of the involved variables on tumor response in patients with HCC suffering TACE were examined by receiver operating characteristic (ROC) curve analysis.

Results: The serum MCP-1 and miR-210 levels were significantly elevated in HCC patients compared to healthy subjects. Patients with the low preintervention MCP-1 and miR-210 levels attained a higher probability of achieving an objective response (OR) (88.5% *vs.*42.9%, P=0.007; 76.9% *vs.* 35.7%, P=0.010, respectively). Pre-TACE MCP-1 level (<816.63 pg/mL) was an independent risk factor associated with OR after TACE by univariate and multivariate analysis while Pre-TACE miR-210 level (<4.43 relative expression) was just positive by univariate analysis. ROC curve analysis showed that a combined index based on those two factors exhibited optimal predictive power of tumor response among all the involved variables (area under the curve =0.823, 95% CI: 0.681–0.965). Additionally, high pre-TACE serum MCP-1 level was correlated with cirrhosis, vascular invasion and Barcelona Clinic Liver Cancer (BCLC) stage. Elevated pre-TACE serum miR-210 level was associated with and BCLC stage.

Conclusions: The study demonstrates that the pre-TACE serum MCP-1 level serves as an effective predictor for tumor response. These findings probably help discriminate HCC patients pre-TACE who specially benefit from TACE regarding OR.

Keywords: Monocyte chemoattractant protein-1 (MCP-1); miR-210; hepatocellular carcinoma (HCC); transarterial chemoembolization (TACE)

Introduction

Transarterial chemoembolization (TACE) is the most frequently applied local-regional treatment, and an approved first choice therapy for the management of BCLC B hepatocellular carcinoma (HCC) (1-3). Surgical resection is regarded as the only radical treatment of HCC. However, the 5-year recurrence rate after hepatectomy reaches approximately 70% (3). Therefore, investigation to improve the survival of HCC patients after radical resection is essential. TACE is a treatment modality resulting from tumor ischemic necrosis due to arterial embolization. TACE has shown improved survival rates in patients after radical resection (3). However not all HCC patients will benefit from TACE in terms of tumor response (4). Thus, how to choose patients who may benefit from TACE treatment appears urgent. To achieve the goal, great efforts have been made in recent years (5,6). For patients with huge tumor size, dispersion distribution or impaired liver function, TACE may not be the first choice treatment. For patients who do not benefit from TACE, early identification of serum markers seems particularly important for they can be conveniently and noninvasively examined at different time period compared with tumor biopsy or imaging-based metrics.

Monocyte chemoattractant protein-1 (MCP-1; CCL2) is a member of the C-C chemokine family which has been shown to be a therapeutic target for the treatment of injury and infection disease (7). MCP-1 also plays important roles in tumorgenesis, metastasis, recurrence, angiogenesis, epithelial-mesenchymal transition (EMT), biomarker and drug tolerance of HCC. Kim *et al*. found that Yap-dependent MCP-1 expression could facilitate the normal liver growth by prohibiting macrophage infiltration in hepatocytes by Hippo signal path (8). MCP-1 can activate Hh and TGF-β pathways in HCC cells to promote metastasis (9,10). MCP-1 was an independent predictor for postoperative recurrence of HCC and overall survival (11). High level of MCP-1 will help angiogenesis and metastasis of HCC by FGFR3 signal path (12). Hedgehog signaling path promotes HCC invasion and EMT *in vitro* by MCP-1/CCR2 axis (13). Moreover, former investigations have demonstrated that MCP-1 level is an encouraging tumor biomarker in HCC (14). MCP-1 induced tumor-associated neutrophils recruit macrophages and T-Regulatory cells to promote resistance to Sorafenib (15). However, few studies focused on MCP-1 levels in HCC patients receiving TACE treatment which is pensive of inflammation and the immune reaction for its hypoxic tumor microenvironment.

MicroRNAs (miRNAs) are a family of evolutionarily conserved non-coding RNAs. MicroRNA-210 (miR-210) is recognized as the major miRNA induced by hypoxia, involved in multiple processes in the hypoxia pathway. For HCC, it plays an important role in cancer angiogenesis, metastasis (16,17). Zhan *et al*. performed a retrospective study and found that after TACE miR-210 changed at different time points closely associated with tumor progression (18).

Thus, we carried out a prospective observational study and sought to testify whether pre-TACE MCP-1 and miR-210 serum levels can act as a predictor of tumor response in patients of HCC suffering TACE. We present the following article in accordance with the MDAR checklist (available at http://dx. doi. org/10.21037/tcr-20-2791).

Methods

Patients and study design

The prospective observation study was conducted in Jiang Su Cancer Hospital from October 2018 to July 2019. This study was registered in the Chinese Clinical Trial Register (registration number ChiCTR-1800017781). The purpose of the study was to determine the role of preintervention MCP-1 and miR-210 serum levels in tumor response of HCC patients received TACE therapy. HCC diagnosis was conducted with the guideline of the European Association for the Study of the Liver (19). Patients enrolled should meet criteria as the following: (I) TACE was the initial treatment; (II) Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0–1; (III) no serious infection before TACE; (IV) Child-Pugh classification A or B. Exclusion criteria: (I) Failed to get informed consent; (II) Multiple cancers; (III) Lack of histological diagnosis; (IV) No follow-up information. A total of 40 HCC patients were involved in the investigation. Sample size calculation was

Table 1 Baseline characteristics of the hepatocellular carcinoma of study population

Characteristics	HCC (n=40)
Sex, male/female	37/3
Age, mean ± SD, years	59.4±10.6
Liver cirrhosis, absence/presence	19/21
Maximum tumor size, mean ± SD, cm	6.8±3.9
Vascular invasion, absence/presence	26/14
AST, median (range), U/L	42.5 (8.0–227.0)
ALT, median (range), U/L	36.0 (17.0–153.0)
ALB, median (range), g/dL	41.5 (29.1–49.5)
TBIL, median (range), μmol/L	13.0 (5.1–138.3)
AFP, median (range), μg/mL	49.2 (1.4–14,966.0)
Child-Pugh classification, A/B	38/2
BCLC stage, B/C	24/16
ECOG PS, 0/1	14/26
HBsAg, absence/presence	3/37

HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALB, albumin; TBIL, total bilirubin; AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBsAg, hepatitis B surface antigen.

based on the preliminary experiment using PASS Software (NCSS, LLC, USA). In the pre-experiment, MCP-1 was measured from 13 HCC patients. The ORR rate was 51.61%. MCP-1 achieve a sensitivity of 80% at a specificity of 87.5%. The sample size was chosen in order to ensure a power of 0.8, with a type I error threshold of 0.05. The minimum sample size of 20 patients was calculated based on sensitivity, while at least 33 patients were calculated based on specificity. Therefore, a total of 40 patients were selected for the study. Patient demographic was presented in *Table 1*. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The protocol for this study and the use of patient blood samples were approved by the ethics committee of the JiangSu Cancer Hospital (2018-050). All patients agreed to participate and signed a written consent.

Measurement of serum MCP-1 concentration

HCC patient serum samples were collected before TACE, while for the control group, were collected form 17 healthy volunteers, and then separated the serum by centrifugation at 3,000 rpm for 10 minutes within 2 hours. Samples were stored at –80 degrees for further examination. Serum MCP-1 concentration was measured by ELISA using the Human monocyte chemotactic protein 1 ELISA kit (ab179886, abcam, USA). Absorption at 450 nm was measured using a microplate reader (Bio-Rad, USA). A standard curve was set up for ELISA (using a four-parameter logistic curve fit). The absorbance values were transferred into the software GraphPadPrism and samples concentrations were back-calculated using the standard curve fitted with four parameters. The detection range for serum MCP-1 ELISA was 4.7 to 300 pg/mL with a sensitivity of 1.26 pg/mL and inter-assay variabilities of <10%. Serum samples were diluted 10-fold in PBS and each dilution was then subjected to ELISA assay. Three biological repeats were performed, in triplicate. The linear regression (r^2) of our curve was closely linear with an average of r^2 >0.97.

Quantification of miR-210 expression levels

Total RNA was extracted from serum using Trizol (Invitrogen, USA) following the manufacturer's protocol. Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed on the extracted RNA from the serum samples. The primers for miR-210 were 5'-ACACTCCAGCTGGGCTGTGCGTGTGAC AGCGG-3'and 5'-CTCAACTGGTGTCGTGGA-3'. The real time RT-PCR was performed according to the following reaction condition: 10 min at 95 ℃ followed by 50 cycles of 15 s at 95 ℃ and 1 min at 60 ℃. miR-210 relative expression was examined using the delta–delta cycle threshold value ($2^{-\Delta\Delta Ct}$) method relative to the U6 as the endogenous control. RT-PCR analysis was performed on a [RT-PCR machine]. All PCR reactions were replicated three times.

TACE and evaluation of tumor response

All enrolled patients underwent imaging with a contrast-enhanced CT or MR imaging protocol before and after 4–6 weeks of the initial TACE. TACE procedures were performed by two interventional radiologist (with over 10 years of experience in HCC interventions) followed by standardized approach. Tumor response was evaluated by three experienced radiologists according to the modified Response Evaluation Criteria in Solid Tumors (mRECIST). OR means patients with objective response (OR), including

complete or partial remission (CR + PR). Non-OR (non-responding), represents patients in the condition of stable or progressive disease (SD + PD).

Statistical analyses

Statistical analysis was performed by SPSS version 25.0 and GraphPad Prism version 8.0 software. Differences between groups were determined by non-parametric Mann–Whitney U test or *t*-test for continuous variables. Chi-square tests or Fisher's exact test were used for categorical data. Receiver operating characteristic (ROC) was constructed to identify the ability of the potential factors for discriminating HCC patients or predicting tumor response. The cut-off values were assessed according to the highest Youden index. Logistic regression was applied to analyze the multivariate variables with tumor response. Statistical significance was considered as P<0.05.

Results

Baseline characteristics of the HCC study population.

In our study, we included 40 HCC patients treated with TACE. The baseline characteristics of the HCC study population were shown in *Table 1*. The mean age of the patients was 59.4 years. Nearly 92.5% were men. The mean maximum tumor size was 6. 8 cm. Among the patients, about half (19/40, 47.5%) patients with liver cirrhosis. Most patients (38/40, 95%) were in Child-Pugh classification A with good liver function. Based on the Barcelona Clinic Liver Cancer (BCLC) classification, 60% and 40% of the patients had the stage of B and C, respectively. 87.5% of the enrolled patients had a good ECOG PS (0–1).

Serum levels of MCP-1 and miR-210 were elevated in HCC patients with TACE compared to healthy controls.

The serum concentration of MCP-1 by ELISA assay was significantly elevated in patients with HCC patients compared to healthy controls (*Figure 1A*). At the same time, RT-qPCR and analysis via the $2^{-\Delta\Delta Ct}$ method revealed increased expression levels of miR-210 in the HCC patients when compared with healthy controls (*Figure 1B*). Mean MCP-1 concentrations for the HCC patients were higher relative to healthy controls (617.62 *vs.* 131.47 pg/mL, *Figure 1A*, Table S1), while mean miR-210 levels for the HCC patients was 4.62 relative to healthy controls (*Figure 1B*,

Table S1). ROC analyses suggest that serum MCP-1 and miR-210 expression levels distinguish HCC patients from healthy controls with an area under the curve of 0.918 (95% CI: 0.848–0.988) and 0.872 (95% CI: 0.772–0.972), respectively (*Figure 1C,D*).

Preintervention serum MCP-1 and miR-210 levels predict tumor response after TACE

Our data showed that serum levels of MCP-1 and miR-210 can distinguish HCC patients from healthy controls. And then, we further explored the predictive value of preinterventional serum levels for the response to TACE therapy. According to the modified RECIST criteria (20), we divided our patients into two groups. One group is OR, means patients with OR, including complete or partial remission after TACE for 4–6 weeks. The control group is non-OR (non-responding), represents patients in the condition of stable or progressive disease after TACE for 4–6 weeks. Our data shows that the OR patients after TACE has the lower level of preintervention serum MCP-1 and miR-210 compared to the Non-OR patients (*Figure 2A,B*). Consistent with this, the AUC values of MCP-1 and miR-210 serum levels can distinguish between OR and non-OR patients by ROC curve analysis (0.739, 0.698, respectively; *Figure 2C*). The 95% confidence interval for MCP-1 and miR-210 distinguish OR from non-OR patients were (0.574–0.904) and (0.516–0.880) respectively. At the threshold of 816.63 pg/mL, MCP-1 sensitivity was 57. 1% and specificity was 88.5%, whereas miR-210 sensitivity was 64. 3% and specificity was 76.9% at the threshold of 4.43 (Table S2). As shown in Table S3, patients with low preintervention MCP-1 and miR-210 serum levels have more opportunity to have OR after TACE (88.5% *vs.* 42.9%, P=0.007; 76.9% *vs.* 35.7%, P=0.010, respectively). Based on our results, we took the preintervention MCP-1 and miR-210 serum levels for the combined diagnostic factor. The formula followed on the two variables logistic regression analysis. Combined preintervention MCP-1 and miR-210=−2.068+2.273* (if preintervention MCP-1 serum levels >816.63 pg/mL) +1.734* (if preintervention miR-210 serum levels >4.43 relative expression) (Table S4). Combined the preintervention MCP-1 and miR-210 serum levels can significant improve the sensitivity and little decrease the specificity (85.7%, 68.2%, respectively, Table S2). Then, we explored which can predict the best value of tumor response in HCC Patients undergoing TACE. ROC curves indicated that combined the preintervention MCP-
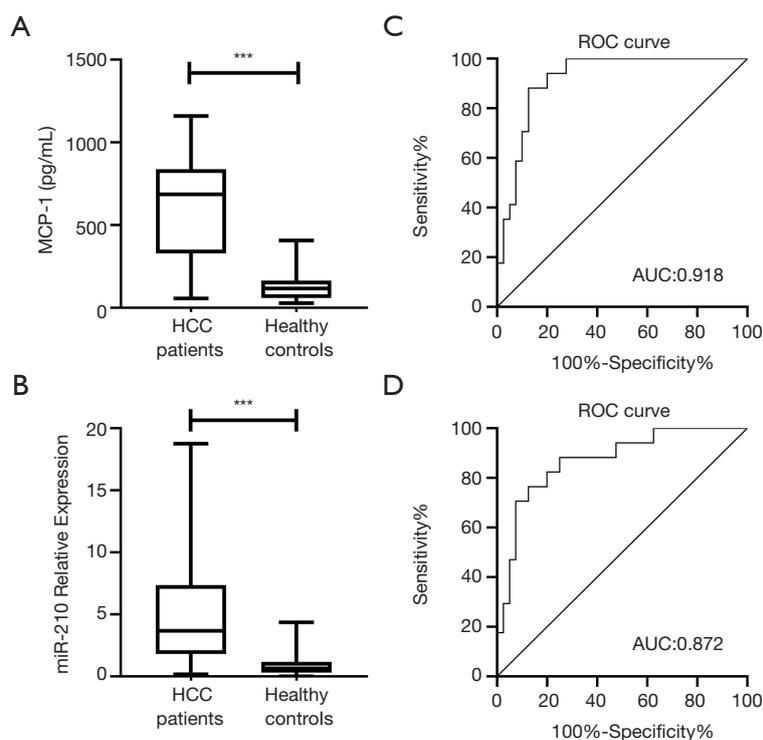
Figure 1 Serum levels of MCP-1 and miR-210 were elevated in HCC patients with TACE compared to healthy controls. (A) Mean expression levels of serum MCP-1 was determined by ELISA analysis; (B) miR-210 were determined by RT-qPCR and analyzed using the delta–delta cycle threshold value (2-ΔΔCt) method relative to the healthy controls. The midline in box plots represents the median, and the upper and lower limits depicting the maximum and minimum values, respectively. (C,D) ROC curves were estimated for MCP-1 and miR-210.AUC indicated 0.918 and 0.872 for MCP-1 and miR-210 regarding the discrimination between HCC patients with TACE and healthy controls. N=40 and 17 for TACE patients and healthy controls, respectively. ***, P<0.001. MCP-1, monocyte chemoattractant protein-1; TACE, transarterial chemoembolization; HCC, hepatocellular carcinoma; miR, microRNA; ROC, receiver operating characteristic; AUC, area under the curve; HCC, hepatocellular carcinoma.

1 and miR-210 serum levels had the best prediction value of tumor response (AUC =0.823; 95% CI: 0.681–0.965) than MCP-1 (AUC =0.739 95% CI: 0.574–0.904) or miR-210 (AUC =0.698; 95% CI: 0.516–0.880) alone. Other indicators including AFP (AUC =0.563; 95% CI: 0.390–0.770), BCLC stage (AUC =0.522; 95% CI: 0.332–0.712), and Child-Pugh classification (AUC =0.571; 95% CI: 0.377–0.766; *Table 2*). To investigate the potential predictors of TACE therapy, chi-square test was used to assess the parameters. We observed that liver cirrhosis (P=0.015), the preintervention MCP-1 serum levels (P=0.007), and the preintervention miR-210 serum levels (P=0.010) were significantly associated with OR (*Table 3*). And then, for multivariate analysis, we found the preintervention MCP-1 serum levels was an independent risk factors for OR (P=0.035; 95% CI: 1.143–43.275; *Table 4*). We did not

conclude the scoring systems of Child-Pugh classification or BCLC stage mainly avoiding the interference of collinearity factors. These scoring systems can be drawn from the variables such as vascular invasion and serum albumin etc.

Associations of preintervention MCP-1 and miR-210 serum levels in patients baseline characteristics

To further explore the associations between serum levels and patients' baseline characteristics, we carried out the chi-square test to examine the relationship. The results presented that preintervention MCP-1 level correlated with vascular invasion (P=0.007), liver cirrhosis (P=0.002) and BCLC stage (P=0.025, *Table 5*). Meanwhile, miR-210 level was found to correlate with vascular invasion (P=0.010) and BCLC stage (P=0.008, *Table 6*).
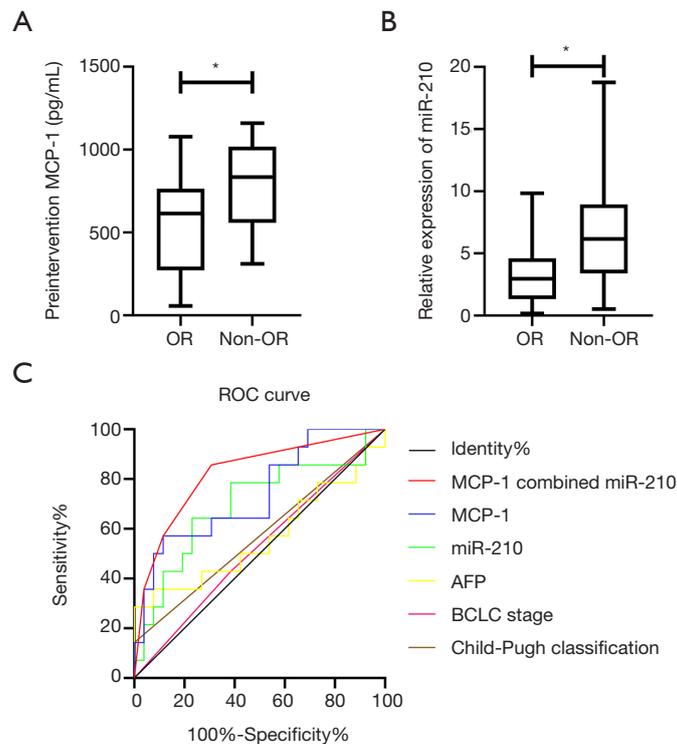
Figure 2 Preintervention MCP-1 and miR-210 serum levels predict tumor response after TACE. (A,B) Compared to the Non-OR patients, OR patients has lower preintervention serum MCP-1 and miR-210 levels. The midline in box plots represents the median, and the upper and lower limits depicting the maximum and minimum values, respectively. (C) ROC curves analysis distinguish the OR and Non-OR patients. N=26 and 14 for OR and non-OR to HCC patients treated with TACE, respectively. *, P<0.05. MCP-1, monocyte chemoattractant protein-1; TACE, transarterial chemoembolization; Non-OR, non-responding; miR, microRNA; OR, objective response; ROC, receiver operating characteristic; HCC, hepatocellular carcinoma.

Table 2 Discriminant abilities of the variables examined

Variables	AUC	P value	OR (95% CI)
AFP	0.580	0.411	0.390–0.770
BCLC stage	0.522	0.821	0.332–0.712
Child-Pugh classification	0.571	0.461	0.377–0.766
MCP-1	0.739	0.014	0.574–0.904
miR-210	0.698	0.041	0.516–0.880
MCP-1 combined miR-210	0.823	0.001	0.681–0.965

AUC, area under the curve; OR, objective response; BCLC, Barcelona Clinic Liver Cancer; MCP-1, monocyte chemoattractant protein-1.

Discussion

Increasing evidence indicates a key role of MCP-1 in the process of carcinogenesis, progression, metastasis in HCC. This study is the first to demonstrate the predictive role of the preintervention MCP-1 level in tumor response of patients with HCC undergoing TACE. We found that serum MCP-1 level in HCC patient increased significantly contrast with healthy individuals which is in accordance with Wang's study (14). Pre-TACE MCP-1 level (<816.63 pg/mL) was an independent risk factor associated with OR after

Table 3 Characteristics Associated tumor response with OR and non-OR group

Characteristics	OR (n=26)	non-OR (n=14)	P value
Sex (male/female)	25/1	12/2	0.571
Age (≤50/>50 years)	5/21	1/13	0.578
Maximum tumor size (≤5/>5 cm)	14/12	7/7	0.816
Vascular invasion (absence/presence)	18/8	8/6	0.677
Liver cirrhosis (absence/presence)	16/10	3/11	0.015
Baseline ALB (≤35/>35 g/dL)	3/23	3/11	0.710
Baseline TBIL (≤17/>17 mmol/L)	18/8	6/8	0.104
Baseline AFP (≤400/>400 µg/L)	19/7	8/6	0.501
Preintervention MCP-1 Serum Levels (≤816.63/>816.63 pg/mL)	23/3	6/8	0.007
Preintervention miR-210 Serum Levels (≤4.43/>4.43 relative expression)	20/6	5/9	0.010
HBsAg (absence/presence)	2/24	1/13	1.000

OR, objective response; ALB, albumin; TBIL, total bilirubin; AFP, alpha-fetoprotein; MCP-1, monocyte chemoattractant protein-1; miR, microRNA; HBsAg, hepatitis B surface antigen.

Table 4 Multivariate Logistic regression analyses for tumor response

Variables	Multivariate analysis	
	OR (95% CI)	P value
AFP	0.295–9.838	0.551
Liver cirrhosis	0.579–19.970	0.176
MCP-1	1.143–43.275	0.035
miR-210	0.854–25.120	0.075

OR, objective response; AFP, alpha-fetoprotein; MCP-1, monocyte chemoattractant protein-1; miR, microRNA.

TACE. The pre-TACE MCP-1 level seems to be an effective predictor of OR in HCC. We hold the option that pre-TACE MCP-1 level (>816.63 pg/mL) may represent immune and inflammatory response before treatment, after chemoembolization hypoxia tumor microenvironment will aggravate immune and inflammatory response which is a poor prognosis for HCC patients. CC chemokines, especially MCP-1 is major attractant of monocyte and macrophage precursor to the tumor microenvironment. Thus, MCP1 can be regarded as biomarkers of inflammation. After TACE, hypoxia elicits inflammation response. It has documented that the systemic inflammation response index can predicting prognosis in HCC patients. It is a better predictor of OS than AFP and Child stage. It appeared superior to clinical Child stage and AFP level in prognosis prediction (21).

One explanation probably lies in inflammatory factors play a crucial role in promoting proliferation, invasion and metastasis of malignant cells. MCP-1 plays important roles in tumorgenesis, metastasis, recurrence, angiogenesis, EMT, biomarker and drug tolerance of HCC. In addition, tumor-resident MSCs expressed high levels of CCL2/MCP-1 promoting tumor growth by recruiting monocytes/macrophages (22). Functionally, experimental studies have demonstrated that MCP-1 induced by VEGF participate in angiogenesis, promoting macrophage recruitment, collagen deposition, and tumor recurrence (23). Moreover, persistent activation of the MCP-1/CCR2 signaling pathway is detrimental to the patient, which may result in EMT metastasis (13,24). Highly expressed MCP-1 level can inhibit cellular immunity by helping T cells differentiate into TH2 cells but not TH1 cells (25). MiR-210 known as the major miRNA induced by hypoxia also increased evidently because of HCC hypoxia microenvironment (26). Distinctly elevated miR-210 was proved to be another significant predictor for OR in our study. Specially, a new criterion based on the pre-TACE MCP-1 level was found to display the best predictive role among all the variables measured. Nevertheless, MCP-1 combined with miR-210 presented better predictive power of tumor response.

Meanwhile, the data demonstrated that elevated pre-TACE plasma MCP-1 level was correlated with vascular invasion, cirrhosis, and BCLC stage. The explanation may be that the elevating MCP-1 level was closely related to HCC

Table 5 Associations between preintervention MCP-1 serum levels and patients' characteristics

Characteristics	Preintervention MCP-1 serum levels		P value
	Low level (n=29)	High level (n=11)	
Sex (male/female)	27/2	10/1	1.000
Age (≤50/>50 years)	5/24	1/10	0.882
Maximum tumor size (≤5/>5 cm)	17/12	4/7	0.208
Vascular invasion (absence/presence)	23/6	3/8	0.007
Liver cirrhosis (absence/presence)	17/12	2/9	0.022
Baseline ALB (≤35/>35 g/dL)	4/25	2/9	1.000
Baseline TBIL (≤17/>17 mmol/L)	19/10	5/6	0.427
Baseline AFP (≤400/>400 µg/L)	20/9	7/4	1.000
Baseline AST (≤40/>40 U/L)	15/14	3/8	0.302
Baseline ALT (≤40/>40 U/L)	18/11	6/5	0.942
Child-Pugh classification (A/B)	28/1	10/1	0.479
BCLC stage (B/C)	21/8	3/8	0.025
ECOG PS (0/1)	8/21	6/5	0.221
HBsAg (absence/presence)	2/27	1/10	1.000

MCP-1, monocyte chemoattractant protein-1; ALB, albumin; TBIL, total bilirubin; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBsAg, hepatitis B surface antigen.

Table 6 Associations between preintervention miR-210 serum levels and patients' characteristics

Characteristics	Preintervention miR-210 serum levels		P value
	Low level (n=25)	High level (n=15)	
Sex (male/female)	24/1	13/2	0.642
Age (≤50/>50 years)	3/22	3/12	0.819
Maximum tumor size (≤5/>5 cm)	15/10	6/9	0.220
Vascular invasion (absence/presence)	20/5	6/9	0.010
Liver cirrhosis (absence/presence)	14/11	5/10	0.165
Baseline ALB (≤35/>35 g/dL)	2/23	4/11	0.253
Baseline TBIL (≤17/>17 mmol/L)	15/10	9/6	1.000
Baseline AFP (≤400/>400 µg/L)	19/6	8/7	0.257
Baseline AST (≤40/>40 U/L)	14/11	4/11	0.071
Baseline ALT (≤40/>40 U/L)	14/11	10/5	0.505
Child-Pugh classification (A/B)	24/1	14/1	1.000
BCLC stage (B/C)	19/6	5/10	0.008
ECOG PS (0/1)	7/18	7/8	0.231
HBsAg(absence/presence)	2/23	1/14	1.000

miR, microRNA; ALB, albumin; TBIL, total bilirubin; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Performance Status; HBsAg, hepatitis B surface antigen.

progression and hepatic stellate cells up-regulates CCR2 by down-regulation of miR-19b-3p to promote liver fibrosis (27-29). High levels of miR-210 are reciprocally associated with BCLC stage which is in line with previous study (18).

The m-RECIST response is known to all that assessment of OR is crucial in the evaluation of the effect of anti-tumor treatment in HCC (30). The response rates after TACE diverge due to inadequately defined response criteria and various clinicopathologic features of patients. Obviously, the method can't anticipate the tumor response. Our study may provide an easily and noninvasively way to predict the tumor response.

The study has several limitations. Firstly, the study was carried out in a single center with a limited sample size. Secondly, only MCP-1 and miR-210 levels were detected in serum without HCC tissues. Thirdly, the relative level of miR-210 was measured but not the absolute concentration. The methods to examine the absolute concentration such as conjugated magnetic beads are needed (31). Finally, OS was not evaluated in the present study because of the limited follow-up.

In a word, the present investigation suggests that pre-TACE serum levels of MCP-1 acts as an early candidate marker for anticipating OR in TACE-treated patients with HCC. These findings may be useful in choosing patients who may benefit from TACE. Evidently, MCP-1 combined with miR-210 exhibited optimal predictive power of tumor response. A multi-center study with a huger number of patients and longer observation time is necessary for testing the results.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the MDAR checklist. Available at http://dx. doi.org/10.21037/tcr-20-2791

Data Sharing Statement: Available at http://dx. doi.org/10.21037/tcr-20-2791

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx. doi.org/10.21037/tcr-20-2791). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013), and approved by the ethics committee of the JiangSu Cancer Hospital (2018-050). All patients agreed to participate and signed a written consent. This study was registered in the Chinese Clinical Trial Register (registration number ChiCTR-1800017781).

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Table S1 Serum levels of laboratory markers

Variables	TACE patients mean (range)	Healthy controls mean (range)
MCP-1 (pg/mL)	617.62 (57.35–1,158.87)	131.47 (27.57–406.27)
miR-210 (Relative expression)	4.62 (0.17–18.76)	1.00 (0.00–4.36)

TACE, transarterial chemoembolization; MCP-1, monocyte chemoattractant protein-1; miR, microRNA.

Table S2 Discriminant abilities of the variables examined

Variables	Sensitivity	Specificity	Youden index	Optimal cut-off values
MCP-1	57.1%	88.5%	0.456	816.63
miR-210	64.3%	76.9%	0.412	4.43
MCP-1 combined miR-210	85.7%	68.2%	0.549	−1.74

MCP-1, monocyte chemoattractant protein-1; miR, microRNA.

Table S3 Preintervention MCP-1 and miR-210 serum levels with objective response after TACE

Characteristics	OR	non-OR	P value
Preintervention MCP-1 Serum Levels			0.007
Low (≤816.63 pg/mL)	23 (88.5%)	6 (42.9%)	
High (>816.63 pg/mL)	3	8	
Preintervention miR-210 Serum Levels (≤4.43/>4.43 relative expression)			0.010
Low (≤4.43 relative expression)	20 (76.9%)	5 (35.7%)	
High (>4.43 relative expression)	6	9	

MCP-1, monocyte chemoattractant protein-1; TACE, transarterial chemoembolization; OR, objective response; miR, microRNA.

Table S4 Multivariate logistic regression models of preintervention MCP-1 and miR-210 serum levels with tumor response

Variables	B	Wald	P value	95% CI
MCP-1	2.273	6.561	0.010	1.705–55.234
miR-210	1.734	4.429	0.035	1.126–28.457
Constant	−2.068	10.061		

MCP-1, monocyte chemoattractant protein-1; miR, microRNA.