The value of KRAS gene status in predicting local tumor progression of colorectal liver metastases following radiofrequency ablation

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ABSTRACT

Purpose: We investigated the relationships between KRAS gene status and local tumor progression (LTP) of colorectal liver metastases (CLMs) after treatment with percutaneous ultrasound-guided radiofrequency ablation (RFA).

Materials and methods: Clinical and imaging data from 76 patients (154 lesions) with CLM who underwent percutaneous ultrasound-guided RFA and had KRAS gene test results between January 2012 and June 2016 were analyzed. The average lesion size was 2.3 ± 1.0 cm (range 0.9–5.7 cm); 38 cases (82 lesions) had wild-type KRAS, and 38 cases (72 lesions) had KRAS mutations.

Results: The technique effectiveness was 98.1% (151/154), and the LTP rate was 18.2% (28/154) after RFA, which was performed between January 2012 and November 2017. The median follow-up was 32.7 ± 2.5 and 32.0 ± 2.6 months (range 1–70 months), respectively. Cumulative LTP rates at 6 months and 1, 2 and 3 years post-RFA for all patients were 7.4, 14.5, 17.8 and 19.2%, respectively. The LTP rate for patients with mutant KRAS (27.8% [20/72]) was significantly higher than that in patients with wild-type KRAS (9.8% [8/82]; p = .004). The cumulative LTP rates at 6 months and 1, 2 and 3 years post-RFA were 4.0, 11.1, 11.1 and 11.1%, respectively, for patients with wild-type KRAS and 11.2, 18.4, 25.2 and 36.2%, respectively, for patient with mutant KRAS (p = .011). Univariate (p = .011) and multivariate analyses (p = .005) showed that KRAS genotype in liver metastases was predictive of LTP. Multivariate analysis also showed that ablation margin size (p< .001) and modified clinical risk score (CRS; p = .033) were independent prognostic factors for LTP.

Conclusions: KRAS gene status of liver metastatic lesions was associated with LTP rates after RFA of CLM. Ablation margin size and modified CRS were also independent prognostic factors for LTP.

Introduction

The liver is a common metastatic site for colorectal cancer [1], and approximately 20–50% of patients with colorectal cancer have liver metastases [2,3]. Surgical resection is the standard treatment for colorectal liver metastases (CLMs). Due to tumor burden and clinical complications, only 17–20% of patients with CLMs can be treated with surgical resection [4,5]. Tanis et al. [6] compared the resection arm of a randomized controlled trial (RCT) of chemotherapy with the radiofrequency ablation (RFA) arm of a similar RCT and showed that when lesion size was limited to 30 mm, the local recurrence rates of surgical resection and RFA were 5.5 and 2.9% per lesion, respectively. There were no major differences in outcomes between resection and RFA for smaller tumors.

In a previous report, for patients with CLMs, local tumor progression (LTP) after treatment with RFA (10%) was lower than that after wedge resection (19%) [7]. Similarly, in an RCT describing RFA (with or without resection) [8], 119 patients with unresectable CLMs received systemic treatment alone or systemic treatment plus aggressive local treatment by RFA. The results showed that 3-, 5- and 8-year overall survival (OS) rates were 56.9, 43.1 and 35.9%, respectively, in the RFA + chemotherapy group and 55.2, 30.3 and 8.9%, respectively, in the chemotherapy-only group. Moreover, aggressive local treatment prolonged OS, and the LTP rate was higher in the chemotherapy-only group. Their results also suggested that RFA may be an effective treatment for CLM [9,10].

The factors affecting LTP rates have been investigated in patients with CLMs who were treated with RFA. For example, Shady et al. [11] showed that a margin of over 5 mm was critical for local tumor control with tumors ablated. The same investigators [12] indicated that no LTP was observed for tumors ablated with margins over 10 mm, regardless of the use of RFA or microwave ablation. Additionally, Gu et al. [13] proposed that the location of the primary tumor and size of the tumor may be independent prognostic factors for patients with CLMs treated with RFA; patients with left-sided lesions or tumors less than 3 cm in size had low LTP rates.
Other studies [14–16] demonstrated the presence of tumor cells in biopsy samples taken from ablation areas. Sotirchos et al. [14] reported that there was only one residual tumor of 67 original tumors (2%) at the first post-RF ablation contrast-enhanced CT scan, which was performed within 4–8 weeks after ablation. Image-guided biopsy was performed from the tumor center and the suspected minimal margin of the ablation zone using coaxial 18–20-gauge core needles, and 16 viable tumors were identified by immunohistochemical analysis. LTP occurred in 11 (69%) of 16 lesions classified as viable and in 10 (20%) of 51 lesions classified as necrotic. Facciorusso et al. [17] demonstrated that the ratio of lymphocytes to monocytes in patients with CLMs treated with RFA was an important factor affecting LTP. Additionally, Odio et al. [18] found that patients with CLMs who had prior hepatectomy had much lower LTP rates.

KRAS gene status is a potential prognostic marker for patients with CLMs [19,20]. Mutations in KRAS can promote tumorigenesis [21,22], possibly through vascular invasion and hematoogenous metastasis [23,24]. Various studies have described the relationships between KRAS mutations and postoperative LTP rates in patients with CLMs; however, the results have varied. For example, Kemeny et al. [25] and Vauthey et al. [26] showed that patients with KRAS mutations had higher cumulative LTP rates. Others [27] found that patients with KRAS mutations in codon 13 had high rates of extrahepatic recurrence. However, Osumi et al. [28] and Margonis et al. [29] showed that KRAS mutations were not associated with LTP.

Few studies have examined the relationships between gene mutations and LTP post-RFA in patients with CLMs. Odio et al. [18,30] suggested that the RAS gene is an important factor affecting LTP in CLMs after RFA. This is consistent with the results of Shady et al. [31], who found that KRAS mutation is a poor prognostic factor of LTP that becomes extremely important when associated with margins under 5 mm.

Accordingly, the purpose of this study was to analyze the relationships between KRAS gene status and LTP in patients with CLMs treated with image-guided RFA.

**Materials and methods**

**Patient selection and eligibility criteria**

The patient database was searched for those with CLM whose records were created between January 2012 and June 2016. In total, 187 patients with CLM who underwent ultrasound-guided RFA were identified; 13 patients who were treated with open ultrasound-guided RFA were excluded. Among the 174 patients who had percutaneous ultrasound-guided RFA, 93 patients did not have genetic test results. For the remaining 81 patients, KRAS gene status was not available in five patients; therefore, 76 patients (154 lesions) were included in the current analysis (Figure 1).

*Ablation procedure*

The treatment plan was decided by at least three physicians who were RFA specialists. The RFA device was selected based on the size, shape and location of the tumor. Patients received general anesthesia and then underwent ultrasound-guided RFA. All RFA procedures were performed by four radiologists (CMH, YK, WW and YW), who each had more than 10 years of experience in ultrasound-guided interventional procedures. The ablation procedure was performed by two physicians together. One physician operated the ultrasound instrument to locate and guide lesions in real time and performed enhanced ultrasound if necessary; the other physician was the operator, placing the electrode needle into the tumor. In this study, the Celon Lab Power ablation system (Olympus, Germany) was used for 132 lesions, the Valleylab system (Tyco Healthcare, North Haven, CT) was used for 11 lesions, and the RITA Model 1500X ablation system (AngioDynamics, Latham, NY) was used for 6 lesions. The MedSphere system (Medsphere Healthcare Corporation, Shanghai, China) was used for five lesions. In the pre-operative protocol, ablation margins were planned to be at least 5 mm, except for tumors adjacent to major structures, such as the diaphragm, bowel, gallbladder, kidney or large blood vessels. Collateral thermal injury to the adjacent structures is still a technical complication of percutaneous RFA [32]. A prior study showed that hydrodissection is technically feasible and effective for improving protection of the adjacent structures by separating them away from the liver capsule [33,34]. In this study, 62 lesions (42 patients) were subjected to hydrodissection due to their location near the diaphragm, bowel, gallbladder or kidney. Forty lesions (19 patients) were adjacent to the diaphragm, 11 lesions (10 patients) were adjacent to the bowel, four lesions (four patients) were adjacent to the gallbladder, and seven lesions (six patients) were adjacent to the kidney. Patients were closely monitored for any complications postablation. There were no differences in

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*Figure 1. Patient disposition.*
therapies received by patients harboring wild-type or mutant KRAS after RFA.

**KRAS mutation profiling**

The tumor samples were surgically resected from the primary tumor and subjected to hematoxylin and eosin (HE) staining and DNA extraction. KRAS genotype (including codons 12 and 13) was determined by direct sequencing or using spectrometry.

**Assessment of therapeutic efficacy and follow-up**

Enhanced computerized tomography (CT) or magnetic resonance imaging (MRI) and ultrasound of the abdomen within 1 month prior to RFA were performed. All related preprocedural examinations were performed within 1 month prior to RFA. At 1 month after RFA, enhanced CT or contrast-enhanced ultrasonography (CEUS) was performed to determine whether the lesions remained in order to evaluate the effectiveness of RFA. As described in a previous report [35], anatomic landmarks around the tumor were chosen. The pre- and 1-month postablation CT images were reviewed side by side to measure the distances from the edge of the tumors to the landmarks, allowing us to select the minimum of multiple margin values on CT as the minimal margin. The margin size was assessed and categorized as 0–5, 6–10, or >10 mm.

Patients were examined with CEUS, enhanced CT, or MRI every 3 months in the first 2 years after RFA and every 6 months at 2 years after RFA. LTP rates and OS were recorded. The time between the diagnosis of liver metastasis and RFA ranged from 0 to 63 months (mean: 11.2 ± 10.4 months), and the time between the diagnosis of primary cancer and RFA ranged from 3 to 92 months (mean: 16.5 ± 14.1 months).

**Definitions**

Technical efficacy [36] refers to treatment of the tumor and complete replacement by ablation zones, with assessment of tumor coverage during the first enhanced imaging follow-up 4 weeks post-RFA. LTP [36] describes the appearance of tumor foci at the edge of the ablation zones after at least one contrast-enhanced follow-up study has documented adequate ablation and covered by the ablation zone in the target tumor and surrounding ablation margin using imaging criteria. OS [36] was the time from the start of ablation treatment to death or the last follow-up. Major complications [36] were events leading to substantial morbidity and disability (e.g., resulting in unexpected loss of an organ), necessitating increased levels of care, hospital admission or lengthened hospital stay. All other complications were considered minor.

**Statistics analysis**

Statistical analysis was performed using SPSS 19.0 (SPSS Inc., Chicago, IL). In this study, chi-square tests were used to compare the clinical characteristics and LTP rates of patients with wild-type and mutant KRAS. The Kaplan–Meier method and log-rank method were used for LTP analysis and univariate analysis. The variables of interest identified in the univariate analysis were further analyzed in the multivariate analysis using the Cox regression model. OS was analyzed using the Kaplan–Meier method. Variables included in the LTP analysis were as follows: sex and age of the patient, primary site of the tumor, pathological differentiation of the primary lesion, lymph node metastasis, depth of invasion, time of liver metastasis, ablative margin size, carcinoembryonic antigen, clinical risk score (CRS), presence or absence of extrahepatic metastases before RFA, number of liver metastases, history of liver metastasis resection, KRAS genotype and lesion size after treatment with RFA.

**Results**

**Technique effectiveness**

Technique effectiveness was 98.1% (151/154) for lesions treated during the first enhanced imaging follow-up 1 month post-RFA. Residual lesions were found in three lesions (three patients) that were adjacent to blood vessels, the biliary tract, or the gastrointestinal tract. Wild-type KRAS was found in two of three patients. In this study, three of 154 lesions were found to have residual tumors, and many new metastases were found in the liver at 1 month after ablation. The clinician decided to administer systemic chemotherapy without repeated ablation. In contrast, eight of 28 locally progressed lesions were treated with repeated ablation. Moreover, 26.5% (9/34) of patients were found to have new liver metastases within 30 d after ablation. Additionally, 44.4% (4/9) of patients had multiple metastases, and 55.6% (5/9) of patients had solitary metastases. There were no extrahepatic metastases within 30 d after ablation.

**LTP analysis**

Baseline characteristics and clinical data of the 76 (154 lesions) patients with wild-type or mutant KRAS are shown in Table 1. No significant differences were found in the age, sex, location of primary lesions, extrahepatic metastases or time of metastasis between patients with wild-type and mutant KRAS.

The overall LTP rate was 18.2% (28/154) after RFA, and patients were regularly followed up until November 2017. The median follow up was 32.0 ± 2.6 months (range: 1–70 months). The cumulative LTP rates at 6 months, 1 year, 2 years and 3 years post-RFA for all patients were 7.4, 14.5, 17.8 and 19.2%, respectively. The LTP rate was 27.8% (20/72) for mutant KRAS, which was significantly higher than that in patients with wild-type KRAS (9.8% [8/82], \( p = .004 \)). When the ablation margin size of the tumor lesions was less than or equal to 5 mm, the LTP rates for wild-type and mutant KRAS were 44.4% (4/9) and 77.8% (7/9), respectively; the difference was not significant (\( p = .335 \)). When the ablation margin size of the tumor lesions was greater than 5 mm, the LTP rates for wild-type and mutant KRAS were 5.5% (4/73)
and 20.6% (13/63), respectively; the difference was significant ($p = .019$).

The cumulative LTP rates at 6 months, 1 year, 2 years and 3 years post-RFA were 4.0, 11.1, 11.1, and 11.1%, respectively, for patients with wild-type KRAS and 11.2, 18.4, 25.2 and 36.2%, respectively, for those with mutant KRAS; the difference was significant ($p = .011$).

Univariate analysis showed that KRAS genotype in liver metastases ($p = .011$), tumor size ($p = .002$), ablation margin size ($p < .001$) and CRS ($p = .038$) were predictors of LTP (Figure 2(a–d)). Using the multivariate regression model, wild-type KRAS ($p = .005$, hazard ratio [HR] = 0.309), margin size ($p < .001$, HR = 0.180), and modified CRS ($p = .033$, HR = 2.692) were found to be independent prognostic factors of LTP (Table 2). Patients with mutant KRAS, CRS 3–5, and margins of 5 mm or less had higher LTP rates than the patients with wild-type KRAS, CRS 0–2, and margins of greater than 5 mm ($p < .001$).

New liver metastases

New liver metastases were found in 44.7% (34/76) of patients, including 39.5% (15/38) of patients with wild-type KRAS and 50.0% (19/38) of patients with mutant KRAS; the difference was not significant ($p = .356$). The 1-, 2- and 3-year cumulative survival rates in patients without new liver metastases were 63.4, 60.1 and 54.6%, respectively, for patients with wild-type KRAS and 57.0, 53.9 and 40.4%, respectively, for patients with mutant KRAS ($p = .629$). New liver metastases were not identified as important factors affecting LTP ($p = .956$) and OS ($p = .647$). New extrahepatic metastasis was a prognostic factor for OS ($p = .007$).

### Table 1. Baseline characteristics of patients with colorectal liver metastases.

|                          | No. of patients | KRAS gene status | $p$ Value |
|--------------------------|-----------------|------------------|-----------|
|                          | Wild-type       | Mutant           |           |
| Age, years               |                 |                  |           |
| $\leq 60$                | 36              | 16               | 20        | .358 |
| $>60$                    | 40              | 22               | 18        |     |
| Sex                      |                 |                  |           |
| Female                   | 49              | 27               | 22        | .231 |
| Male                     | 27              | 11               | 16        |     |
| Tumor size, cm           |                 |                  |           |
| $\leq 3$                 | 54              | 27               | 27        | .000 |
| $>3$                     | 22              | 11               | 11        |     |
| Primary site of the tumor|                 |                  |           |
| Right colon              | 15              | 5                | 10        | .150 |
| Left colon               | 61              | 33               | 28        |     |
| Pathological differentiation of the primary lesion |  | | |
| poorly                   | 15              | 7                | 8         |     |
| Moderately or well       | 61              | 31               | 30        |     |
| Depth of invasion        |                 |                  |           |
| T1–3                     | 29              | 12               | 17        | .238 |
| T4                       | 47              | 26               | 21        |     |
| Lymph node at the primary site |  | | |
| Positive                 | 58              | 28               | 30        |     |
| Negative                 | 18              | 10               | 8         |     |
| Time of liver metastasis |                 |                  |           |
| Synchronous              | 45              | 22               | 23        | .966 |
| Heterochronous           | 31              | 15               | 16        |     |
| Extrahepatic metastases  |                 |                  |           |
| Yes                      | 40              | 19               | 21        | .646 |
| No                       | 36              | 19               | 17        |     |
| No. of liver metastases  |                 |                  |           |
| $\leq 3$                 | 57              | 26               | 31        | .185 |
| $>3$                     | 19              | 12               | 7         |     |
| CEA                      |                 |                  |           |
| $\leq 30$                | 62              | 31               | 31        | 1.000 |
| $>30$                    | 14              | 17               | 17        |     |
| History of liver metastasis resection pre-RFA |  | | |
| Yes                      | 26              | 12               | 14        | .629 |
| No                       | 50              | 26               | 24        |     |
| Margin, mm               |                 |                  |           |
| $\leq 5$                 | 9               | 4                | 5         |     |
| 6–10                     | 55              | 26               | 29        |     |
| $>10$                    | 12              | 8                | 4         |     |
| CRS                      |                 |                  |           |
| 0–2                      | 30              | 15               | 15        | 1.000 |
| 3–5                      | 46              | 23               | 23        |     |
| DFI                      |                 |                  |           |
| $<12$                    | 16              | 7                | 9         |     |
| $\geq 12$                | 60              | 31               | 29        |     |

RFA: radiofrequency ablation; No: number. Modified CRS: Node-positive primary tumor, DFI <12 months, more than one liver tumor, size of largest tumor >3 cm, CEA level >30 ng/mL (mg/L) (Radiology [11]). DFI: disease-free interval from primary resection to the diagnosis of liver metastasis.
Survival

The 1-, 2- and 3-year survival rates were 89.5, 65.0 and 34.9%, respectively; the median survival time was 32.0 months. KRAS gene status (p = .228) did not predict OS.

Complications

In total, 154 lesions were treated with RFA. Major complications occurred in five patients. Three patients developed hepatic hematoma, which improved after conservative treatment; one patient developed proteinuria and improved after alkalization of the urine; and one patient had abdominal pain, fever and biliary tract infection after ablation. The lesion was adjacent to the hilar bile duct, and the pre-RFA ultrasound showed expansion of the bile duct behind the tumor. Cephalosporin combined with metronidazole was used for treatment. Additional symptoms were alleviated after conservative treatment.

Discussion

RFA is a safe and effective method for the treatment of CLMs [37,38]. Ruers et al. [8] demonstrated a survival benefit with the use of RFA in addition to chemotherapy. However, approximately 12–40% of patients had LTP post-RFA [9,39],
and managing LTP remains a clinical challenge. In this study, we found that KRAS gene status was an independent factor affecting the prognosis of patients with CLMs.

KRAS protein is a basic component of the epidermal growth factor receptor-signaling cascade. Activating mutations in the KRAS gene cause constitutive activation of Ras GTPase, which leads to excessive activation of the downstream Raf/extracellular signal-regulated kinase/mitogen-activated protein kinases and other signaling pathways, thereby resulting in cell transformation and tumorigenesis.

Many studies have reported the relationship between the KRAS gene and LTP in patients who underwent surgical resection. Vincenzi et al. [20] and Vauthey et al. [26] suggested that KRAS mutations could predict the rate of LTP in patients with colorectal cancer who underwent surgical resection. Renaud et al. [40] proposed that KRAS gene status may be used as a predictive marker for patients with colorectal lung metastases who received surgical treatment. In another study [41], analysis of KRAS gene status in 918 patients with CLMs showed that 477 patients had wild-type KRAS and 441 had KRAS mutations, and higher cumulative LTP rates were found at specific sites, such as the brain, bones and lungs, for patients with mutant KRAS.

In this study, we found that patients with KRAS mutations had a higher cumulative LTP rate than patients with wild-type KRAS. Similarly, Calandri et al. [30] reported that mutant

### Table 2. Univariate and multivariate analyses for factors affecting local tumor progression.

| Variables                        | No. of tumors | Univariate analyses | Multivariate analyses |
|----------------------------------|---------------|---------------------|----------------------|
|                                  |               | p Value             | p Value             |
| Age                              |               |                     | Hazard ratio         |
| ≤60                              | 73            | .674                | –                    |
| >60 years                        | 81            | –                   | –                    |
| Sex                              |               |                     |                      |
| Male                             | 98            | .454                | –                    |
| Female                           | 56            | –                   | –                    |
| Tumor size                       |               |                     |                      |
| ≤3 cm                            | 121           | .002                | .697                 |
| >3 cm                            | 33            | –                   | 1.199                |
| Primary site of the tumor        |               |                     | 0.481–2.985          |
| Right colon                      | 21            | 0.437               | –                    |
| Left colon                       | 133           | –                   | –                    |
| Pathological differentiation     |               |                     |                      |
| Poorly                           | 24            | .068                | –                    |
| Moderately or well               | 130           | –                   | –                    |
| Depth of invasion                |               |                     |                      |
| T1-3                             | 65            | .111                | –                    |
| T4                               | 89            | –                   | –                    |
| Lymph node status                |               |                     |                      |
| Positive                         | 114           | .917                | –                    |
| Negative                         | 40            | –                   | –                    |
| KRAS gene                        |               |                     |                      |
| Wild-type                        | 82            | .011                | .005                 |
| Mutation                         | 72            | –                   | 0.309                |
| Time of liver metastasis         |               |                     | 0.135–0.706          |
| Synchronous                      | 108           | .168                | –                    |
| Heterochronous                   | 46            | –                   | –                    |
| Ablative margin (mm)             |               |                     |                      |
| ≤5                               | 18            | –                   | –                    |
| 5–10                             | 108           | .000                | .000                 |
| >10                              | 28            | –                   | 0.180                |
| Extrahepatic metastases          |               |                     | 0.890–0.362          |
| Yes                              | 79            | .991                | –                    |
| No                               | 75            | –                   | –                    |
| DFI (months)                     |               |                     |                      |
| <12                              | 124           | .895                | –                    |
| ≥12                              | 30            | –                   | –                    |
| CEA                              |               |                     |                      |
| ≤30                              | 120           | .109                | –                    |
| >30                              | 34            | –                   | –                    |
| CRS                              |               |                     |                      |
| 0–2                              | 61            | .038                | .033                 |
| 3–5                              | 93            | –                   | 2.692                |
| 6–10                             | –             | –                   | 1.084–6.688          |
| No. of liver metastases          |               |                     |                      |
| ≤3                               | 98            | .689                | –                    |
| >3                               | 56            | –                   | –                    |
| History of liver Metastasis resection pre-RFA | – | .583 | – |
| Yes                              | 49            | –                   | –                    |
| No                               | 105           | –                   | –                    |

RFA: radiofrequency ablation; No: number.
Modified CRS: Node-positive primary tumor, DFI < 12 months, more than one liver tumor, size of largest tumor >3 cm, CEA level > 30 ng/mL (mg/L) (Radiology [11]).

DFI: disease-free interval from primary resection to the diagnosis of liver metastasis.
KRAS was an independent predictor of LTP; patients with mutant KRAS had higher LTP rates than patients with wild-type KRAS. Shady et al. [31] added a significant threshold: a margin of 5 mm was found to be particularly important for KRAS mutant tumors. In the subset of patients with KRAS mutation, a suboptimal margin carried a significant risk (HR: 16.8) for LTP when compared with wild-type KRAS ablated with margins of greater than 5 mm, highlighting the importance of modifying technique based on KRAS status. Adequate safety margins are a critical determinant of LTP after ablation of liver tumors [11,12,14,30]. In this study, margin size was a significant predictor of LTP for CLMs. Consistent with these findings, Shady et al. [12] proposed that a margin of over 5 mm was critical for local tumor control with tumor ablation, a margin of 10 mm is associated with no recurrence, regardless of the use of RFA or MWA. Additionally, Shady et al. [11] demonstrated that if the margins were over 10 mm, local tumor control of over 90% could be achieved at more than 4 years after ablation. Moreover, a previous study [35] revealed that a minimal margin of greater than 5 mm was a critical factor for local tumor control.

In our study, the modified CRS was a predictor of LTP. The modified CRS was determined based on the original surgical CRS, which included node-positive primary tumors, the disease-free interval from primary tumor resection to CLM detection, the number of liver tumors, the size of the largest tumor, and carcinoembryonic antigen (CEA) levels. We defined the modified CRS as described in a previous report [11], in which the modified CRS was also found to be a predictor of LTP. Additionally, Sofocleous et al. [42] indicated that patients with a modified CRS (not including CEA levels) of 0–2 had higher LTP than those with a modified CRS of 3–4 at 1 year after a single ablation. However, Shady et al. [12] demonstrated that there was no significant difference in tumor progression between patients with CRS 0–2 and CRS 3–5. Thus, more studies involving greater numbers of patients are needed to confirm these findings. If the application of such a CRS may help identify patients at high risk, patient outcomes may be improved via appropriate use of follow-up schedules or combined therapies.

There are many factors affecting LTP in patients with CLMs who were treated with RFA [14,35,43], including tumor size, surviving tumor cells, lymphocyte to monocyte ratio and history of liver metastasis resection pre-RFA. In our study, univariate analyses showed that lesion size was predictive of LTP. However, the improvement of ablation instruments has increased the maximum range of ablation. Pre-operative planning, multipoint ablation and multiple needles can be used for large tumors. Technical success can be achieved for tumors that are not located in special sites. Accordingly, lesion size was not found to be an independent predictor, similar to previous findings [12,31], and there were no significant associations between LTP and lesion size.

There were no significant differences in new liver metastases between patients with wild-type KRAS and mutant KRAS post-RFA. This was different from the findings reported by Shady et al. [31]. Other studies [40,41] suggested that KRAS gene status is associated with tumor recurrence at specific sites, such as the lungs, bones and brain. Further studies are needed to elucidate the relationships between the KRAS gene and new liver metastases.

The 1-, 2- and 3-year survival rates were 89.5, 65.0 and 34.9%, respectively, consistent with a recently published report [11]. However, Shady et al. [31] suggested that KRAS gene status was related to OS for CLMs treated with RFA. In contrast, our findings showed that there were no significant differences in OS between patients with wild-type KRAS and mutant KRAS. Therefore, the relationship between KRAS gene and OS in patients with CLMs who received RFA is still unclear and should be studied in greater detail using larger sample sizes.

The KRAS genotype in liver metastases was treated similarly to the genotype of the primary lesions in this study. Loes et al. [44] showed that 13 of 94 patients exhibited mutation heterogeneity across metastatic deposits for at least one gene. However, Voutsina et al. [45] and Tie et al. [46] found that the genes of the primary tumor lesions were highly consistent with the genes of the metastatic sites. Knijn et al. [47] studied 305 patients with colorectal cancer and found that the genes were consistent from the primary tumor lesions and metastatic sites in 98% of patients. Thus, this study considered that the KRAS genotype of the primary tumor and the metastatic sites were the same; further studies on the genotypes of liver metastases have been planned.

This study had some limitations. Although we found that the baseline characteristics of patients with wild-type KRAS and mutant KRAS were comparable and without significant differences, it was difficult to avoid bias since this study was a single-center retrospective study with a small sample size. Second, previous studies have shown that the KRAS gene is associated with metastases at specific sites among patients with CLMs who received surgical treatment. Because this was not explored in this study, various consecutive research projects have been planned to further investigate the relationships between the KRAS gene and specific site metastases.

Finally, BRAF and NRAS genes have been reported to be associated with prognosis in patients with CLMs who underwent surgical treatment [28,48]. In this study, we only analyzed the relationships of the KRAS gene with OS and LTP rate. Relevant data are being collected and analyzed in order to elucidate the associations between other genes and prognosis in patients who treated with RFA.

Conclusions

KRAS gene status in the liver metastatic lesions was found to be associated with the rate of LTP after RFA in CLMs. KRAS mutation status in the ablated lesion significantly predicted LTP. In addition, ablation margin size and modified CRS were also independent prognostic factors for LTP.

Disclosure statement

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