Histopathology of the Tissue Adhering to the Multiple Tine Expandable Electrodes Used for Radiofrequency Ablation of Hepatocellular Carcinoma Predicts Local Recurrence

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Abstract

Objective To assess the ability to predict the local recurrence of hepatocellular carcinoma by analyzing tissues adhering to the radiofrequency ablation probe after complete ablation.

Methods From May 2002 to March 2011, tissue specimens adhering to the radiofrequency ablation probe from 284 radiofrequency ablation sessions performed for hepatocellular carcinomas ≤3 cm in size were analyzed. The specimens were classified as either viable tumor tissue or complete necrosis, and the local recurrence rates were calculated using the Kaplan-Meier method.

Results From the tumors ≤3 cm in size, viable tissue was present in 6 (2.1%) of 284 specimens, and the local recurrence rates after 1 and 2 years of follow-up were 6.7% and 11.2%, respectively. Local recurrence developed significantly earlier in the viable tissue group. The recurrence rate was not significantly different based on whether transcatheter arterial chemoembolization was performed.

Conclusion The histopathology of the tissue adhering to the radiofrequency ablation probes used for hepatocellular carcinoma treatment can predict local recurrence. Additional aggressive treatment for patients with viable tissue can therefore improve the overall survival.

Key words: hepatocellular carcinoma, radiofrequency ablation, local recurrence, viable tissue, electrode

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related mortality worldwide (1, 2), with an estimated 600,000 people dying annually of HCC (3). Surgical resection has been the standard curative treatment for HCC. However, many patients are not candidates for surgery at presentation because of the presence of multifocal tumors, advanced tumors, or poor hepatic functional reserve (4). Liver transplantation provides an alternative curative treatment for small unresectable HCC (5). However, a shortage of liver grafts limits the applicability of this approach (6).

Radiofrequency ablation (RFA) was first described by Rossi et al., and is a local thermal ablation therapy for HCC (7). RFA, a palliative modality for HCC, causes coagulative necrosis and results in cell death (8). Curley et al. advocated the open approach of RFA, and they performed open RFA in 74.8% of 123 patients with unresectable primary or secondary liver malignancies. They reported complete tumor ablation of all treated tumors, with a local recurrence rate of only 1.8% (8). Among the various methods used for local tumor control, RFA is considered to be a promising alternative to surgery (9).

Local tumor recurrence remains an important limitation of
RFA (10). While performing a series of percutaneous RFA of liver tumors, it was noted that tissue always adhered to the probe and its electrodes after the Radiofrequency Interstitial Tumor Ablation (RITA) Medical System (AngioDynamics, Queensbury, NY) was used. It was therefore postulated that it might be feasible to perform a histological assessment of the viability of this tissue, and that the viability could conceivably be used as a predictor of local recurrence. The purpose of this study was to analyze the ability to perform such a histological assessment of the viability of tissues adherent to the RFA probe, and to determine whether or not the results correlated with the local recurrence of HCC after complete RFA.

Materials and Methods

Subjects

From May 2002 to March 2011, tissue specimens were collected from 284 RFA sessions performed for HCC in patients with lesions ≤3 cm in the largest tumor diameter. The patient characteristics are presented in Table. The study was designed to examine the histopathologic characteristics of the tissue specimens extracted from the probe after RFA for HCC. All HCC patients had undergone triphasic computed tomography (CT) of the liver in order to plan the ablation procedure.

Equipment

The radiofrequency (RF) system used in this study was a 460 kHz generator (RITA Medical System Model 500 and 1500X; AngioDynamics) with an expandable electrode needle as previously described (11). The procedure was performed on inpatients under conscious sedation using a combination of 25 mg intramuscular hydroxyzine (Atarax P; Pfizer Japan Inc., Tokyo, Japan) and 15 mg pentazocine (Pentagin; Daiichi Sankyo Company, Ltd., Tokyo, Japan) and 0.5 mg atropine sulfate (Fuso Pharmaceutical Industries, Ltd., Osaka, Japan).

The patients were monitored by pulse oximetry during the entire procedure. A prophylactic antibiotic (1 g of imipenem/cirastatin (IPM/CS), MSD K.K., Tokyo, Japan) was administered intravenously just prior to the procedure. The ablation was performed using a 50 mm RITA Medical System StarBurst XL RF probe (AngioDynamics). This probe was able to generate a 5 cm lesion with a core temperature of about 80°C. In this study, only HCC ≤3 cm in the largest diameter were analyzed. The goal was to create an area of complete necrosis at least 1 cm larger than the tumor diameter in order to achieve a minimum ablation margin around the tumor of 5 mm (12).

Inclusion and exclusion criteria

The inclusion criteria were as follows: 1) age of 18 to 75 years; 2) HCC ≤3 cm in the largest diameter; 3) absence of vascular invasion, lymph node involvement, and distant metastases; 4) liver function status of Child-Pugh A or B; 5) platelet count >50×10^9/L; and 6) the patients were either contraindicated for a partial hepatectomy or refused to undergo the procedure. Patients with intractable ascites and/or uncorrectable coagulopathy were excluded from the study.

Furthermore, based on a report by Nakazawa et al., the analysis was limited to cases in which a sufficient ablated margin was demonstrated on diagnostic imaging following RFA (12).

Ethical considerations

A priori approval of the present study by the appropriate institutional review committee was granted. Before enrolling them in the study, informed consent in writing was obtained from each patient. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Histopathological analysis

Immediately after each RFA session, all tissue fragments that adhered to the RFA electrodes and tines were collected.

<table>
<thead>
<tr>
<th>Table. Patient Characteristics (n = 284)</th>
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<tr>
<td>Age, years (mean ± SD)</td>
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<tr>
<td>Male: Female</td>
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<tr>
<td>Etiology</td>
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<tr>
<td>HCV only 174 (61.3 %)</td>
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<tr>
<td>HBV only 63 (22.2 %)</td>
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<td>Both positive 6 (2.1 %)</td>
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<td>Both negative 41 (14.4 %)</td>
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After collection, all tissue fragments were immediately fixed in 10% formalin, dehydrated, embedded in paraffin, and cut into 5 μm-thick sections and subsequently stained with hematoxylin and eosin (H&E). The specimens were classified by the study pathologists (N.I. and T.K.) as either viable tumor tissue or complete necrosis.

Necrosis was defined as the complete absence of cells or the presence of necrotic or amorphous material. Of note, the cellular staining faded with time toward a “thermal fixation” of the tissue treated with RFA (13). Consequently, small islands of affected “ghost cells” within the regions of coagulative necrosis were not considered to represent viable tissue. In contrast, a diagnosis of “viable” neoplastic cells was based on the evidence of normal hepatocytes and HCC cells that exhibited homogeneous eosinophilic staining of the cytoplasm and dense basophilic H&E staining of the nuclei (14).

Staining with Ki-67, a cell proliferation marker, was also performed (monoclonal mouse clone MIB-1; Dako, Denmark) in six nodes in which residual viable cells were diagnosed. The specimens were classified as having low (<10%), medium (<20%) or high (≥20%) Ki-67 labeling, and were also investigated with regard to local recurrence.

**Imaging follow-up**

Triphasic or portal venous phase contrast-enhanced CT was performed within seven days of the procedure and used to evaluate the response to treatment. A lack of enhancement of the ablated tumor was considered to be evidence of complete and technically successful ablation. Irregular peripheral or nodular enhancement within 1 cm of the ablated area, especially in the portal venous phase, was considered to represent an untreated (residual) tumor and thus was considered to be a technical failure (15). Thereafter, radiological follow-up continued at three-month intervals for at least one year and was used to help evaluate local tumor recurrence. Evidence of irregular or nodular enhancement within 1 cm of the previously treated tumor was considered to be local tumor recurrence.

**Statistical analysis**

Local recurrence of HCC was analyzed using the Kaplan-Meier technique (16), and differences in the curves were tested using the log-rank test. The tumor size was expressed as the means and standard deviation. For all of the analyses, a P value <0.05 was considered to be statistically significant. The data were analyzed using a commercially available software program (Statview, Stata; College Station, TX).

**Results**

During the study period, 284 HCC tumors ≤3 cm in size were treated with RFA. Representative samples of viable tumor and complete necrosis are shown in Fig. 1. The patient characteristics are summarized in Table. In all RFA sessions, there was no evidence of residual tumor on the first post-RFA contrast-enhanced CT.

Tissue adhering to the electrode was obtained in all cases, thus resulting in 284 specimens that were subjected to a histological analysis. All tissue fragments were collected and examined by the study pathologist. In six (2.1%) of the 284 specimens of HCC ≤3 cm, non-coagulation necrosis (viable tissue) was present.
Local recurrence

The median follow-up period was 28.1 months (range, 2.2-43 months). The local recurrence rates of HCC ≤3 cm in diameter were 6.7% and 11.2% at 1 and 2 years after RFA, respectively (Fig. 2). The local recurrence rates were not statistically significantly different based on the use of transcatheter arterial chemoembolization (TACE) (Fig. 3). The 1-year primary local recurrence rates were 50.0% and 5.7% in the viable tumor and complete necrosis groups, respectively (Fig. 4). The 2-year primary local recurrence rates were 75.0% and 8.6% in the viable tumor and complete necrosis groups, respectively (Fig. 4). Local recurrence occurred significantly earlier in the viable tumors group than in the complete necrosis group.

A representative case of local recurrence is presented in Fig. 5. With regard to Ki-67 immunohistochemical staining of the six viable tissue specimens, local recurrence was observed in three of the four low labeling index specimens and the one medium labeling index specimen, but was not observed in the one high labeling index specimen. A preoperative biopsy was not performed, but neither the infiltrative type nor the simple nodular type with extranodular growth was observed on diagnostic imaging.

Discussion

RFA was first used for the treatment of HCC in humans in 1993 (7) after extensive animal studies (17, 18). The goal of the therapy was to ablate tumor tissue percutaneously with RF energy delivered directly through a non-insulated electrode tip. RFA has since emerged as a popular local ablative therapy for unresectable HCC because of its efficacy and safety.

RFA with a percutaneously inserted electrode can ablate tumors more completely than other locoregional treatments, and can thus reduce the rate of local recurrence (19, 20). Complete ablation of HCC is required for prevention of local recurrence and a good prognosis (21). For small HCC ≤3 cm in size, initial complete tumor response rates have been reported to be ≥90%, and local recurrence rates have been reported to be 10-20% (20, 22). However, there was one report of local recurrence rates at RFA sites as high as 36% (23). Local recurrence after successful ablation of HCC using RFA is therefore an important issue. Unfortunately, incomplete tumor ablation (24) and local tumor recurrence or progression remain problematic (23, 25).

Tissue viability immediately after radiofrequency ablation with a RITA probe in the normal pig liver was evaluated using RFA is therefore an important issue. Unfortunately, incomplete tumor ablation (24) and local tumor recurrence or progression remain problematic (23, 25). Tissue viability immediately after radiofrequency ablation with a RITA probe in the normal pig liver was evaluated using H&E staining. A core of heat-coagulated tissue was seen in specimens that were not stained by H&E, suggestive of 100% cellular destruction (26). Tissues adherent to the radiofrequency probe after ablation can be examined pathologically and may show coagulation necrosis (26). Given the fact that the histopathologic analysis of tissues adhering to the RFA probe after the procedure is feasible (27) and that complete necrosis can be immediately detected after the treatment (27), the objective of the present study was to assess the value of this post-procedural histological analysis as a possible predictor of local recurrence. This information could then be used to determine whether additional treatment should be offered without waiting for the results of a
Larger tumors are more difficult to completely ablate, thus increasing the risk for local recurrence and who might benefit from early follow-up imaging study.

The present evaluation was limited to tissues adhering to the RFA electrode. Such tissue is expected to be necrotized, owing to direct contact with the heat produced by the electrode and tines. The tumor size has already been reported to be directly related to local tumor recurrence after RF ablation of HCC (28). A prior series reported a local recurrence rate of 4% at the 1-year follow-up for small tumors (<2.5 cm in diameter), while for larger tumors (>2.5 cm in diameter) the 1-year local recurrence rate was five times higher, at 21% (29). Komorizono et al. also reported that for tumors measuring >2 cm in size, the presence of subcapsular lesions was a risk factor for local tumor recurrence (30). Larger tumors are more difficult to completely ablate, thus making it more likely that residual malignant cells are present after the procedure, and thus leading to an increased rate of recurrence.

In the present study, the local recurrence rates were 6.7% and 11.2% at 1 and 2 years after RFA, respectively. Local recurrence occurred significantly earlier in the viable group than in the complete necrosis group, although the local recurrence rates were not significantly different with regard to the use of TACE.

Heat-induced coagulation necrosis constitutes the primary tissue change caused by RFA; however, the reliable determination of live or dead cells in coagulation necrosis may be difficult with standard H&E staining. Ki-67 is well known as a MIB-1 (anti-Ki-67 antibody) index that demonstrates the degree of proliferation. Known to correlate well with signs of neoplastic malignancy and prognosis, including the degree of specialization, blood vessel invasion and lymph node metastasis, in tumors such as breast, stomach and bowel cancer, Ki-67 is a widely used and extremely useful cell proliferation marker. With regard to the Ki-67 immunohistochemical staining of the six tissue specimens with visible cells, local recurrence was observed in three of the four low labeling index specimens and in the one medium labeling index specimen, but not in the one high labeling index specimen. It is therefore possible that the degree of Ki-67 staining is lower for HCC than for other cancer types.

Overall, the H&E staining findings in the present study contributed to the identification of local recurrence, but further examination is required using alternative immunohistochemical staining.

The information obtained from histopathological tissue examinations may be used to help identify patients at high risk for local recurrence and who might benefit from early follow-up and additional treatment despite imaging findings that are negative for local recurrence. The present study demonstrated that the histopathological characteristics of tis-

Figure 5. CT images (pre-RFA, post-RFA, at recurrence) of a representative case with local recurrence. The patient was an 82-year-old woman with primary biliary cirrhosis and hepatocellular carcinoma. (A) A CT scan revealed solitary hepatocellular carcinoma at segment VII before TACE and RFA. (left) Plain CT (middle) arterial phase (right) portal phase. (B) A CT scan obtained 1 month after TACE and RFA showed no sign of local recurrence. The complete ablative margin exceeded the lipiodol region developed by TACE. (left) Plain CT (middle) arterial phase (right) portal phase. (C) A CT scan obtained 12 months after TACE and RFA showed signs of local recurrence at the posterior border ablation zone with enhancement (arrowhead) in the arterial phase (middle) and washout in the portal phase (right). (left) Plain CT (middle) arterial phase (right) portal phase.
sues adherent to the RF electrode after ablation of HCC provides evidence of residual viable tumor cells and is a useful predictor of local recurrence. The presence of viable tumor cells on the RFA electrode should be considered a high-risk finding in HCC.

In order to prevent local recurrence prior to obtaining imaging evidence of any such recurrence, and given the preliminary results of this study and the increased risk of local recurrence for patients in the viable group, an appropriate clinical protocol based on the characteristics of tissues adhering to the RFA probe could thus be developed. This protocol could then be used to guide decisions regarding repeat ablation or other additional treatments (such as embolization, radio-embolization, local arterial chemotheray or systemic treatment). This approach may improve the clinical outcomes in patients with HCC who undergo treatment with RFA. Further prospective trials concerning the histological analysis of RFA are needed.

The authors state that they have no Conflict of Interest (COI).

References


