Tumor Vascular Micro-environment of Colorectal Hepatic Metastasis and Chemotherapy Response

Hirotaka Okamoto (hirotakaoka@hotmail.com)
Tsuru Municipal Hospital
https://orcid.org/0000-0003-2964-5606

Shugo Shiba
Tsuru Municipal Hospital

Hiroshi Iino
Kofu Municipal Hospital

Hiroyuki Wakana
Kofu Municipal Hospital

Daisuke Ichikawa
Yamanashi Daigaku - Igakubu Campus: Yamanashi Daigaku Igakubu Daigakuin Sogo Kenkyubu Iguuki

Research Article

Keywords: Metastatic hepatic tumor, Colorectal cancer, Tumor vascular microenvironment, Angiogenesis, Chemotherapy response, Ring enhancement on CT

DOI: https://doi.org/10.21203/rs.3.rs-448775/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Background: Tumor vascular micro-environment has an important role in tumor progression and metastasis. The objective of this study was to assess the significance of metastatic hepatic tumor vascular micro-environment in relation to the response to the systemic 5-FU-based chemotherapy (FOLFOX or FOLFIRI).

Methods: A total of 48 consecutive colorectal cancer (CRC) patients with hepatic metastasis were retrospectively reviewed, and factors, such as metastatic tumor vascular micro-environment, chemotherapy response, and hepatic resection, were analyzed. Tumor angiogenesis was microscopically evaluated by microvessel density (MVD) in sections stained immunochemically with CD34 in patients with hepatic resection. The angiogenesis in tumor micro-environment in association with ring enhancement (RE) on computed tomography (CT) was also examined.

Results: Microscopic examination revealed that peripheral RE on CT of the metastatic tumor is associated with tumor angiogenesis by MVD. The overall response rate after 6 courses of first-line chemotherapy for the liver metastasis with RE on CT was 64% (23/36), whereas the response rate without RE was 25% (3/12), which was significantly different, although the survival rate of patients with RE-positive or without RE-negative tumors was not different.

Conclusion: The peripheral RE on CT of the metastatic hepatic tumor was associated with the angiogenesis in tumor micro-environment and higher chemotherapy response.

Introduction

Colorectal carcinoma (CRC) is one of the most common cancers. Approximately 50% of patients develop liver metastases at some point during their disease course. Patients who are candidates for surgical resection of their liver metastases can expect a prolonged survival or even cure. However, only 10 to 25% of patients are candidates for liver resection. In patients with unresectable metastases, chemotherapy is the treatment of choice, and although it is often used with palliative intent, it may also be used in an attempt to render the metastases resectable. Chemotherapy can also be administered as a neoadjuvant treatment for selected cases of resectable metastases. Thus, most patients receive chemotherapy.

Tumor vascular micro-environment as angiogenesis is an essential process in many physiological and pathological conditions, including embryonic development, organ regeneration, chronic inflammation, and tumor growth and metastasis. Metastatic liver tumors express angiogenic factors, such as vascular endothelial growth factor (VEGF), which is a well-known angiogenic growth factor. The amount of this angiogenic factor affects patient survival with metastatic disease. In tumor micro-environment, newly formed angiogenic vessels supply the metastatic tumor with nutritional blood and growth factors, which leads to tumor proliferation, growth, and survival.
Imaging, such as computed tomography (CT), is generally used to monitor chemotherapy according to the new guidelines for response evaluation (RECIST criteria)\textsuperscript{14}). Complete response is usually defined as the disappearance of target lesions on imaging and is considered a good indicator to evaluate the efficacy of chemotherapy. The correlation between imaging and pathological status is not well defined. Whether radiological features, including complete radiological response, are correlated with pathological features, including complete pathological response, is important for the management of patients.

We examined the significance of tumor peripheral ring enhancement (RE) on contrast CT in hepatic metastasis from CRC regarding the response to first-line systemic chemotherapy. Furthermore, the correlation between the radiological and pathological status, including the angiogenesis in tumor microenvironment, in patients who underwent resection of the site of the initial liver metastasis was investigated.

**Methods**

**Patients and Methods**

Between 2006 and 2015, data of 48 consecutive patients treated for liver metastasis from CRC were collected prospectively. The treatments were performed at the Department of Gastrointestinal Surgery at Yamanashi University Hospital between 2006 and 2009 or at the Department of Surgery at Tsuru Municipal Hospital between 2010 and 2015.

No patients with synchronous liver metastasis simultaneously underwent resection of both the primary and secondary metastatic lesions. All patients first underwent operation of the primary tumor. Open colectomy was performed in 29 patients and anterior resection of the rectum was performed in 16 patients, including 2 cases of transient colostomy because of cancer ileus. Three patients underwent Mile’s operation. After the initial operation, adjuvant systemic chemotherapy following the 5-FU-based regimen, as described in the systemic chemotherapy section, was started within one month in cases of synchronous metastatic disease. In patients without confirmed hepatic metastasis, prophylactic systemic chemotherapy was not performed. Twelve cases of metachronous hepatic metastasis were found during the follow-up period. After confirmation of newly formed hepatic metastasis, adjuvant systemic chemotherapy was started. Adjuvant chemotherapy was performed for all patients with hepatic metastasis as systemic disease to prevent surrounding invasion or distant metastasis and to increase their resectability. Hepatic resection was conducted excluding inoperable multiple bi-lobular deposits of the liver and/or systemic disease such as lung metastasis or peritoneal dissemination.

**Regimen of Systemic Chemotherapy**

Most patients in this series (n = 48) were treated using a chemotherapy regimen based on fluorouracil (5-FU)/leucovorin (LV) combined with oxaliplatin (mFOLFOX6\textsuperscript{15}) or with irinotecan (FOLFIRI\textsuperscript{16}). The following first-line chemotherapy regimens were administered before the liver metastases disappeared: 39 patients received m-FOLFOX6; 3 patients received m-FOLFOX6 + bevacizumab; 5 patients...
received FOLFIRI and 1 patient received FOLFIRI+panitumumab, for a total of 48 patients (Table 1). Second-line chemotherapy was used in cases of disease progression after first-line chemotherapy. A radiological response to systemic chemotherapy was assessed according to RECIST criteria 14).

**Imaging**

Triple-phase helical CT with 5-mm reconstruction (TOSHIBA TSX-101A, TOSHIBA, Tokyo, Japan) and abdominal ultrasound (TOSHIBA Nemio SSA550A, Xario SSA660A, TOSHIBA, Tokyo, Japan) was performed for all patients before chemotherapy and after every six cycles of chemotherapy. Scans of the liver were acquired with 16x0.75-mm collimation and pitch of 1.356, and were subsequently reconstructed at 3-mm intervals. Settings were 200 mA and 120 kV. Nonionic contrast medium (Omnipaque; Daiichi Pharmaceutical Co, Ltd, Tokyo, Japan) was injected at a rate of 3 mL/s (2 mL/kg) by an MCT power injector. Arterial-phase abdominal images were obtained 35 seconds after injection and portal-phase images were obtained at 80 seconds. Although colorectal liver metastasis was visualized better on the portal venous phase, tri-phasic CT was performed systematically for the evaluation of colorectal metastasis to improve the CT readings. All CT images were reviewed independently by radiologists. The CT images were compared with previous CT images. Abdominal angiography was also performed via a femoral approach by radiologists for some patients 17).

For quantitative analysis of CT images, regions of interest (ROI) were selected on the basis of the best tumor image on the portal phase of contrast-enhanced CT. Tumor peripheral RE was assessed as follows: the ROI in the peripheral RE area was measured by at least five independent enhanced spot areas as a CT-value (Hounsfield Unit: HFU). The CT-value of normal liver parenchyma was also measured at the area nearby the tumor without tumor-altered vascularity. The mean CT-value was used, and the difference in the CT-value between the RE area and the normal liver parenchyma was defined as the RE CT-value (Fig. 3A). The cut-off value between RE positive or negative was defined as 5HFU.

**Tumor Vascular Micro-environment by Pathological and Immunohistochemical Examination**

Resected specimens of liver samples were fixed in 10% formalin and embedded in paraffin. Thin sections were deparaffinized twice with xylene and rehydrated in a series of ethanol solutions. Sections were placed in 0.01 mol/L trisodium citrate dehydrate buffer (pH 6.0) and treated in a microwave oven for 10 min at 500 W.

For CD34 staining, tissue sections were digested with 0.2% trypsin in 0.01 mol/L phosphate-buffer saline for 20 min at 37°C. The tissues were immersed in 3% H₂O₂ with distilled water for 10 min to inactivate endogenous peroxidases. After blocking nonspecific binding by normal goat serum, sections were incubated overnight at 4°C with mouse anti-monoclonal CD34 antibody (1:25; QB-END/10, Novo-castra Laboratories, Newcastle, United Kingdom) as the primary antibody. This was followed by reacting with biotinylated anti-immunoglobulin and labeling using streptavidin-biotin reaction kit peroxidase (Dako,
under light microscopy (200X magnification). Microvessel density (MVD) was used to evaluate the microscopic tumor angiogenesis in colorectal liver metastases \textsuperscript{18}). For MVD after CD34 staining, the average was calculated in the five most peri-tumor vascular areas in the 14 metastatic liver cancer lesions examined at 200X magnification (Fig. 2).

**Patient Management and Follow-Up**

Preoperative systemic chemotherapy was continued postoperatively for six to eight cycles except in cases of grade 3 or 4 toxicity. Patients were followed up every 3–4 months during the first 2 years and every 6 months thereafter. At each follow-up visit, tumor recurrence was assessed by clinical examination and liver ultrasound. Abdominal and chest CT was performed every 3–6 months. All surviving patients were followed for a minimum of 12 months after surgery.

**Statistics**

Quantitative data were expressed as the mean and standard deviation. Quantitative and qualitative variables were compared using the Fisher’s exact test or the Mann-Whitney U test as appropriate. Overall survivals in both metachronous and synchronous cases were defined as the period from the starting day of systemic chemotherapy at the development of metastatic hepatic tumors to the day of death from any cause. The survival rate was calculated using the Kaplan-Meier method and the log-rank test was used to assess the survival differences between groups. Significance was defined by a $P$-value $<0.05$.

**Results**

**Patients and Tumor Characteristics**

Clinical and tumor characteristics of 48 patients with liver metastases are shown in Table \textbf{2}. Of these 48 patients, 36 had synchronous metastatic disease and 12 had metachronous disease. The mean age for patients at the time of diagnosis was 66.2 ± 12 years (range 38–82 years). Primary cancers included colon carcinomas in 29 patients and rectal carcinomas in 19 patients. TNM classification \textsuperscript{19}) was as follows: T1-T2 in 2 and T3-T4 in 46. There was lymph node involvement in 38 patients. Pathological diagnoses included well-differentiated adenocarcinoma in 27, moderate differentiated adenocarcinoma in 20, and mucinous adenocarcinoma in 1. The mean number of liver metastases was 4.8 (range 1–12). Distant metastases excluding hepatic metastases were observed in 11 patients with lung metastasis on preoperative CT examination. Peritoneal dissemination was confirmed in 7 patients by surgical exploration.

Thirty-six patients had synchronous hepatic metastasis. Thirty-one synchronous cases were initially treated by resection of the primary lesion followed by systemic chemotherapy, and 6 patients eventually underwent liver resection. The other 30 patients were not considered for resection of the liver metastases for the following reasons: inoperable multiple bi-lobular deposits of the liver and/or systemic disease.
colostomy for colon obstruction. After confirmation of a good chemotherapy response, the primary lesion was removed.

Twelve of 48 metachronous patients had hepatic metastasis. Metachronous hepatic metastasis was defined by a hepatic tumor found longer than 12 months after the initial treatment. There were 8 resectable metastatic liver tumors and 4 inoperable cases. Hepatic resection was eventually performed in 14 patients, including 42 lesions. The operative procedures included hemihepatectomy (n = 2), segmentectomy or sectionectomy (n = 6), and partial resection (n = 21). Additional ablation therapy by radio frequent ablation (RFA) was performed in 10 patients.

Clinical tumor characteristics of liver metastases with or without RE on CT are summarized in Table 2. There was no significant difference between the two groups regarding the clinical characteristics except for pathological diagnosis.

**Initial Chemotherapy Responses**

Overall chemotherapy responses are shown in Table 3. The total response rate was 54% (26/48), including 2 in CR and 24 in PR. Chemotherapy responses separated by metastatic hepatic tumor characteristics with or without peripheral RE are indicated. The chemotherapy response rate of RE-positive tumors was 64% (23/36) and that of RE-negative tumors was 25% (3/12), which was significantly different. The disease control rate (CR + PR + SD/CR + PR + SD + PD) of RE-positive tumors was 86% (31/36) and that of RE-negative tumors was 75% (9/12).

**Quantitative Analysis of Angiogenesis in Tumor Microenvironment and RE of Metastatic Hepatic Tumors**

Tumors with peripheral RE on contrast-enhanced CT, as shown in Fig. 1A, (thick and thin arrows) corresponded to round stained tumors on abdominal angiography (thick and thin arrows, respectively), as shown in Fig. 1B, suggesting the identification of RE of the hepatic metastatic lesion to newly formed blood flow.

Histopathological and immunostaining examination of a metastatic hepatic tumor is shown in Fig. 2. A microscopic view (40X magnification) of the metastatic hepatic tumor revealed moderately differentiated tubular adenocarcinoma (A). The surrounding tissues, including sinusoidal tissue, hepatocytes, fibroblasts, and endothelial cells, were compressed and invaded by the metastatic hepatic tumor. CD34 staining is shown in B (40X magnification) and C (200X magnification). CD34-stained cells were observed in host liver parenchyma, including compressed sinusoidal tissues, and invaded into metastatic cancer tissues. MVD was measured at peri-tumor areas at 200X magnification using CD34-stained specimens as described in the Methods section.

The relationship between the MVD and CT-value is shown in Fig. 3A and B. The CT-value was also measured as described in the Methods section. MVD was associated with the RE CT-value of the metastatic hepatic tumor, revealing a strong relationship between microscopic tumor angiogenesis and...
the peripheral metastatic tumor RE (correlation coefficient \( r = 0.65 \), p-value = 0.01). The metastatic tumor peripheral RE on CT may reflect angiogenesis of the tumor.

**Overall Survival Rates in Patients with RE-Positive or -Negative Tumors**

The overall survival after systemic chemotherapy between patients with RE-positive and RE-negative tumors is shown in Fig. 4. The overall survival (OS) rate of RE-positive or -negative tumors with hepatic resection is shown in Fig. 4A. There were no significant differences between the groups. The mean OS rate of patients with RE-positive tumors was 42.1 months and that of those with RE-negative tumors was 48.0 months \( (p = 0.6) \). Longer survival was observed in the groups with hepatic resection than without hepatic resection (Fig. 4A,B). The OS rate between patients with RE-positive or -negative tumors without hepatic resection was also not significantly different. The mean OS rate of patients with RE-positive tumors was 25.8 months and that of those with RE-negative tumors was 23.7 months \( (p = 0.8) \). OS rates in all patients were not significantly different between RE-positive tumors and RE-negative tumors (Fig. 4C). The mean OS rate of patients with RE-positive tumors was 28.1 months and that of those with RE-negative tumors was 43.0 months \( (p = 0.05) \).

**Discussion**

We found that the peripheral RE on CT of metastatic hepatic tumors is associated with the angiogenesis in tumor micro-environment and may predict a good chemotherapy response. The combination of liver resection with chemotherapy improved the survival of patients who had multiple hepatic metastases. There are several reports on the concept of peripheral RE on CT of CRC metastasis \(^{20, 21} \). CT-based morphological criteria, including peripheral rim of enhancement of the hepatic metastatic tumor, was reported to have a strong association with the pathological response and survival. These investigations support our study.

Angiogenesis is associated with tumor aggressiveness and poorer prognosis in patients with hepatic tumors\(^{22, 23} \). Tumor angiogenesis facilitates metastatic formation by providing mechanisms to increase the likelihood of tumor cells invading the blood circulation, and provides nutrients for tumor growth and survival at the metastatic site. The interaction of tumor cells with endothelial cells in tumor micro-environment has an essential role in tumor angiogenesis. Blood nutrient supply and tumor-related endothelial cells promote tumor cell proliferation and tumor growth\(^{24} \). The tumor micro-environment is essential for the formation of a newly metastatic lesion. Tumor cells and host-cells, such as endothelial cells or fibroblast, participate in tumor metastasis. Tumors that are not vascularized at the metastatic site are typically maintained as small dormant nodules, and the tumor volume remains constant because of a balance between cell proliferation and cell death. Thus, tumor growth is dependent on angiogenesis.

We assessed clinical angiogenesis of metastatic hepatic tumors using indirect imaging by enhanced CT. The clinical manifestation of peripheral RE on CT of the metastatic hepatic tumor was confirmed to
investigation of the correlation between angiographically assessed vascularity and blood flow in hepatic metastases from colorectal carcinoma\textsuperscript{25}. Of note, the hemodynamics of contrast medium between abdominal angiography and enhanced CT images were different. Angiography images were taken in the direct celiac arterial phase, whereas enhanced CT images were taken in the indirect portal phase through the intravenous injection of contrast medium. However, there was a possibility that tumor staining on angiography and peripheral RE on CT was the same because both images may reflect newly formed angiogenic vessels.

There are several methods to monitor angiogenesis using conventional imaging such as contrast-enhanced US (ultrasound), CT, and MRI (magnetic resonance imaging). Enhanced CT is frequently used in the clinical setting, and can readily access metastatic hepatic tumors and surrounding tissues. Contrast-enhanced CT is useful to evaluate tumor angiogenesis by immunohistochemical quantification of the MVD in colorectal adenocarcinoma patients\textsuperscript{26}. Evaluation of angiogenic vessels by the MVD is associated with microscopic tumor angiogenesis\textsuperscript{18}. We found a strong relationship between microscopic tumor angiogenesis and peripheral metastatic tumor RE on CT.

Hepatic tissue including hepatocytes was fed by blood from the portal vein or hepatic artery, and the blood supply drained via the hepatic vein. Metastatic hepatic tumors may be supplied through angiogenesis via the portal vein or arterial blood flow. Metastatic hepatic tumors were reported to have a dual blood supply from both the portal vein and hepatic artery\textsuperscript{27,28}. In our study, clinical angiogenesis was able to be assessed by not only peripheral RE on portal-phase CT, but also by arterial flow on celiac angiography. This suggested that metastatic hepatic carcinoma was fed from dual blood flow from the portal vein and hepatic artery. Therefore, the clinical manifestation of RE on CT of the metastatic hepatic tumor may be closely associated with tumor angiogenesis.

Angiogenic hepatic metastatic tumors responded well to systemic chemotherapy despite their aggressiveness. Angiogenic tumors may readily uptake anti-cancer drugs to the tumor through newly formed angiogenic vessels. Furthermore, immature angiogenic vessels are fenestrated\textsuperscript{29}. Angiogenic factors, such as VEGF, which was first identified as vascular permeability factor\textsuperscript{30,31}, not only stimulate endothelial cells lining nearby microvessels to proliferate and migrate, but also render these vascular endothelial cells hyperpermeable. Hyperpermeable vessels may readily leak plasma proteins and deliver anti-cancer drugs into the extravascular space.

Recent clinical anti-angiogenic therapies, such as angiogenic antibody, have been used in patients with unresectable metastatic hepatic disease\textsuperscript{32,33}. Anti-angiogenic antibody therapy itself is insufficient for anti-cancer effects. However, a single infusion of anti-VEGF antibody reduced tumor perfusion, vascular volume, microvascular density, interstitial fluid pressure, and circulating endothelial cells in patients with rectal cancer\textsuperscript{34}. This suggests that anti-angiogenic therapy has direct anti-vascular effects on human tumors. A combination of these drugs with anti-cancer drugs produces anti-cancer effects. Anti-angiogenic molecules, such as anti-VEGF antibody, remodel tumor-related endothelial cells into normal endothelial cells with a normal structure\textsuperscript{35,36}. Anti-angiogenic molecules reshape pathologic
vasculature into normal vasculature, which results in delivery of the anti-cancer drug to the tumor. Although we analyzed only a few patients using anti-angiogenic agents in this study, there were anti-tumor effects without anti-angiogenic agents. Anti-angiogenic therapy may not be associated with a direct tumor response, but rather maintenance of anti-tumor effects. Normalization of tumor-related vasculature may enable the sustained delivery of anti-cancer drugs.

Hepatic resection remains the only potential curative treatment for metastatic tumors and improves survival \(^{37,38}\). Similarly, we found that resection of metastatic hepatic tumors improved OS (Fig. 4A, B).

When the groups were divided by hepatic resection, there were no significant differences between patients with RE-positive and -negative tumors with hepatic resection (Fig. 4A). In addition, there were no significant differences between patients with RE-positive and -negative tumors without hepatic resection (Fig. 4B). A higher response rate to systemic chemotherapy was observed in patients with RE-positive tumors, but the OS rate of patients with RE-positive tumors was not significantly different from that of patients with RE-negative tumors (Fig. 4C). This suggests that a higher response to systemic chemotherapy does not always lead to longer survival. After the initial higher response in our patients, additional surgical therapy prolonged survival. To improve survival, additional therapeutic strategies, such as maintenance chemotherapy, use of molecular targeted drugs, or immuno-check point inhibitors, are needed. Clinically, hepatic metastatic tumors can recur or develop other metastatic lesions, such as a lung metastases or peritoneal dissemination, during the follow-up period for RE-positive and -negative tumors, which may affect patient survival.

There were a few limitations in the present study. First, there were patients without clinical manifestations, such as lung metastasis or peritoneal dissemination, during the initial treatment period. Second, the statistical power was weak because the sample size was small. The observational period of 10 years was relatively long, but new molecular drugs, such as anti-VEGF antibody and anti-EGFR antibody, were not frequently used for the initial treatment. Further studies with a larger number of patients and a shorter time period are needed to confirm our results.

**Conclusion**

We found that tumor peripheral RE on contrast CT in metastatic hepatic tumors from CRC was correlated with the angiogenesis in tumor micro-environment and indicated a good response to recent 5-FU-based systemic chemotherapy.

**Abbreviations**

CRC: Colorectal cancer, MVD: Microvessel density, RE: Ring enhancement, CT: Computed tomography, VEGF; Vascular endothelial growth factor, RECIST; Response evaluation criteria in solid tumors, mFOLFOX6; modified fluorouracil (5-FU)/leucovorin (LV) combined with oxaliplatin, FOLFIRI; Fluorouracil (5-FU)/leucovorin (LV) with irinotecan, ROI; Regions of interest, HFU; Hounsfield Unit, RFA; Radio frequent
Declarations

Acknowledgements

None

Authors’ contributions

HO, SS, HI, HW participated in the operation or management of the patient in this study. HO conducted majority of the experiments and wrote the manuscript. DI is the chairperson of our department. All authors have read and approved the final manuscript.

Funding

None

Availability of data and materials

This was not applicable to this manuscript.

Ethics approval and consent to participate

All procedures were in accordance with the ethical standards of the responsible institutional and national committees on human experimentation and with Helsinki Declaration of 1964 and its later versions. Informed consent was obtained from all patients. This article does not contain any studies by any of authors on human or animal subjects.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ. Cancer statistics, 2005. CA. Cancer J Clin. 2005;55:10–30.
2. Stangl R, Altendorf-Hofmann A, Charnley RM, Scheele J. Factors influencing the natural history of colorectal liver metastases. Lancet. 1994;343:1405–10.
3. Leonard GD, Brenner B, Kermeny NE. Neoadjuvant chemotherapy before liver resection for patients with unresectable liver metastases from colorectal carcinoma. J Clin Oncol. 2005;23:2038–48.

4. Scheele J, Stang R, Altendorf-Hofmann A, Paul M. Resection of colorectal liver metastases. World J Surg. 1995;19:59–71.

5. Fortner JG, Silva JS, Cox EB, Golbey RB, Gallowitz H, Maclean BJ. Multivariate analysis with liver metastases from colorectal cancer: Treatment by hepatic resection. Ann Surg. 1984;199:306–16.

6. Scheele J. Hepatectomy for liver metastases. Br J Surg. 1993;80:274–6.

7. Petrelli NJ, Abbruzzese J, Mansfield P, Minsky B. Hepatic resection: The last surgical frontier for colorectal cancer. J Clin Oncol. 2005;23:4475–7.

8. Meric F, Patt YZ, Curley SA, Chase J, Roh MS, Vauthey JN, Ellis LM. Surgery after downstaging of unresectable hepatic tumors with intra-arterial chemotherapy. Ann Surg Oncol. 2000;7:490–5.

9. Adam R, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, Giacchetti S, Paule B, Kunstlinger F. Ghémard O, Levi F, Bismuth H: Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: A model to predict long-term survival. Ann Surg 2004;240:644–57.

10. Allen PJ, Kemeny N, Jamagin W, DeMatteo R, Blumgart L, Fong Y. Importance of response to neoadjuvant chemotherapy in patients undergoing resection of synchronous colorectal liver metastases. J Gastrointest Surg. 2003;7:109–15.

11. Folkman J. angiogenesis in cancer, vascular rheumatoid and other disease. Nat Med. 1995;1:27–31.

12. Risau W. Mechanisms of angiogenesis. Nature. 1997;386:671–4.

13. Onogawa S, Kitadai Y, Tanaka S, Kuwai T, Kimura S, Chayama K. Expression of VEGF-C and VEGF-D at the invasive edge correlates with lymph node metastasis and prognosis of patients with colorectal carcinoma. Cancer Sci. 2004;95:32–9.

14. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubenstein L, Verweij J, Glabbeke MV, Oosterom AT, Christian MC, Gwyther SG. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst. 2000;92:205–16.

15. de Gramont A, Figer A, Seymour M, Homerin M, Hmissi A, Cassidy J, Boni C, Cortes-Funes H, Cervantes A, Freyer G, Papamichael D, Bail NL, Louvet C, Hendler D, de Braud F, Wilson C, MorvanF, Bonetti A. Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol. 2000;18:2938–47.

16. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L. Rougier P:Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: A multicentre randomised trial. Lancet. 2000;355:1041–7.

17. Millis S, Doppman JL, Dunnick NR, McCarthy DM. Evaluation of angiography in Zollinger-Ellison syndrome. Radiology. 1979;131:317–20.

18. Eefsen RL, Engelholm L, Willemoe GL, Eynden GG, Laerum OD, Christensen IJ, Rolff HC, Høyery-Hansen G, Osterlind K, Vainer B, Illemann M. Microvesel density and endothelial cell proliferation
levels in colorectal liver metastases from patients given neo-adjuvant cytotoxic chemotherapy and bevacizumab. Int J Cancer. 2016;138:1777–84.

19. TNM Classification of Malignant Tumours. 7th Edt. Edited by Leslie S, Mary G, Christian W, 2009.

20. Chun YS, Vauthey JN, Boonsirikamchai P, Maru DM, Kopetz S, Palavecino M, Curley SA, Abdalla EK, Kaur H, Chamsangavej C, Loyer EM. Association of computed tomography morphologic criteria with pathologic response and survival in patients treated with bevacizumab for colorectal liver metastasis. JAMA. 2009;302:2338–44.

21. Karaosmanoglu AD, Onur MR, Ozmen MN, Akata D, Karaaltincaba M. Magnetic resonance imaging of liver metastasis. Semin Ultrasound CT MR. 2016;37:533–48.

22. Pang RW, Joh JW, Johnson PJ, Monden M, Pawlik TM, Poon RT. Biology of hepatocellular carcinoma. Ann Surg Oncol. 2008;15:956–71.

23. Sun B, Zhang S, Zhang D, Du J, Guo H, Zhao X, Zhang W, Hao X. Vasculogenic mimicry is associated with high tumor grade, invasion and metastasis, and short survival in patients with hepatocellular carcinoma. Oncol Rep. 2006;16:693–8.

24. Okamoto H, Ohigashi H, Nakamori S, Ishikawa O, Imaoka S, Mukai M, Kusama T, Fujii H, Matsumoto Y. Akedo H. Reciprocal function of liver tumor cells and endothelial cells. Eur Surg Res. 2000;32:374–9.

25. Yamaguchi A, Taniguchi H, Kunishima S, Koh TY, Yamagishi H. Correlation between angiographically assessed vascularity and blood flow in hepatic metastases in patients with colorectal carcinoma. Cancer. 2000;89:1236–44.

26. Goh V, Halligan S, Daley F, Wellsted DM, Guenther T, Bartram CI. Colorectal tumor vascularity: quantitative assessment with multidetector CT-Do tumor perfusion measurements reflect angiogenesis? Radiology. 2008;249:510–7.

27. Taniguchi H, Daidoh T, Shoaki Y, Takahashi T. Blood supply and drug delivery to primary and secondary human liver cancers studied with in vivo bromodeoxyuridine labeling. Cancer. 1993;71:50–5.

28. Casillas S, Dietz WD, Brand MI, Jones SC, Vladisavljevic A, Milsom JW. Perfusion to colorectal cancer liver metastases is not uniform and depends on tumor location and feeding vessel. J Surg Res. 1997;67:179–85.

29. Cameliet P, Jain RK. Principles and mechanisms of vessel normalization for cancer and other angiogenic diseases. Nat Rev Drug Discov. 2011;10:417–27.

30. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science. 1983;22:219:983–5.

31. Dvorak HF, Brown LF, Demar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol. 1995;146(5):1029–39.

32. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Barton A, B, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus
33. Saltz LB, Clarke S, Diaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzen F, Cassidy J. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol. 2008;26:2013–9.

34. Willett CG, Bucher Y, diThomas E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY, Jain RK. Direct evidence that bevacizumab has antivascular effects in human rectal cancer. Nat Med. 2004;10:145–7.

35. Tong RT, Boucher Y, Kozin SV, Winkler F, Hicklin DJ, Jain RK. Vascular normalization by vascular endothelial growth factor receptor 2 blockade induces a pressure gradient across the vasculature and improves drug penetration in tumors. Cancer Res. 2004;64:3731–6.

36. Jain RK. Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. Science. 2005;7:307:58–62.

37. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA:A Cancer J Clin. 2011;61:69–90.

38. Choti MA, Sizmann JV, Tiburi MF, Sumetchotimetha W, Rangsin R, Shulick RD, Lillemoe KD, Yeo CJ, Cameron JL. Trends in long-term survival following liver resection for hepatic colorectal metastases. Ann Surg. 2002;235:759–66.

Tables

Due to technical limitations, table 1 to 3 is only available as a download in the Supplemental Files section.

Figures
Figure 1

A, Metastatic hepatic tumors with ring enhancement (RE) from colorectal cancer. B, Selective angiography from the common hepatic artery. Thick and thin arrows on the CT images correspond to thick and thin arrows on angiography, respectively.
Figure 2

Microscopic examination of a resected hepatic metastatic tumor. Microscopic view (40X magnification) of the metastatic hepatic tumor revealed moderately differentiated tubular adenocarcinoma (A). CD34 staining is shown in B (40X magnification) and C (200X magnification, the square area in B). CD34 was stained as described in the Methods section.
Figure 3

A, Quantitative analysis of the CT findings. The regions of interest (ROI) were selected based on the best tumor image on contrast-enhanced CT. The ROI of the peripheral RE area was measured by at least five independent enhanced spot areas (red cross) as a CT-value (Hounsfield Unit: HFU). The mean CT-value was used, and the difference in the CT-value between the RE area and liver parenchyma area (blue cross) was defined as the RE CT-value. B, The relationship between the MVD and CT-value. MVD was measured using a CD34-stained specimen and CT-values were measured at the peripheral RE area as described in the Methods section.
Figure 4

A, Overall survival (OS) rates in patients with RE-positive or -negative (RE(+) or RE(-)) tumors with hepatic resection. B, OS rates in patients with RE(+) or RE(-) tumor without hepatic resection. C, OS rates in all patients with RE(+) or RE(-) tumors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- JSOTable.pptx