MACC1 mRNA Levels Predict Cancer Recurrence After Resection of Colorectal Cancer Liver Metastases

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Objective: Upon colon cancer metastasis resection in liver, disease outcome is heterogeneous, ranging from indolent to very aggressive, with early recurrence. The aim of this study is to investigate the capability of metastasis associated in colon cancer 1 (MACC1) levels measured in liver metastasis specimens to predict further recurrence of the disease.

Methods: Gene expression and gene dosage of MACC1, hepatocyte growth factor (HGF), and hepatocyte growth factor receptor (MET) were assessed using quantitative realtime polymerase chain reaction on a cohort of 64 liver metastasis samples from patients with complete follow-up of 36 months and detailed clinical annotation. The most relevant mutations associated to prognosis in colorectal cancer, KRAS, and PIK3CA were assessed on the same specimens with Sanger sequencing.

Results: Receiver operating characteristic (ROC) analysis revealed that MACC1 mRNA abundance is a good indicator of metastatic recurrence (AUC = 0.65, P < 0.05), whereas no such results were obtained with MET and HGF, nor with gene dosage. Generation of MACC1-based risk classes was capable of successfully separating patients into poor and good prognosis subgroups [hazard ratio (HR) = 5.236, 95% confidence interval (CI) = 1.2068–22.715, P < 0.05]. Also KRAS mutation was significantly associated with higher risk of recurrence (HR = 2.07, 95% CI = 1.048–4.09, P < 0.05). Cox regression multivariate analysis supported the independence of MACC1, but not KRAS, from known prognostic clinical information (Node Size HR = 1.4418–6.905, P < 0.01). Preoperative carcinoembryonic antigen HR = 2.359, 95% CI = 1.0203–5.452, P < 0.05, MACC1 HR = 7.2739, 95% CI = 1.6584–31.905, P < 0.01).

Conclusions: MACC1, a new easily detectable biomarker in cancer, is an independent prognostic factor of recurrence after liver resection of colorectal cancer metastasis.

Keywords: biomarker, cancer recurrence, colorectal cancer, liver metastasis, MACC1, prognosis


The most frequent site of metastasis from colorectal cancer is the liver: up to 25% of the patients present with liver metastases at the first diagnosis of colorectal cancer, whereas another 20% will develop these metastases after treatment of the primary tumor.1 If feasible, resection of hepatic lesions is the only potentially curative therapy, resulting in 3-year survival rates of up to 60%.2,3 Nevertheless, tumor recurrence after curative resection remains a major problem, usually occurring within the first 3 years after surgery.4 The use of peroperative chemotherapy seems to achieve an approximate 8% to 10% increase in disease-free survival (DFS) rates at 3 years.5–7 Recently, an European expert panel has recommended that most patients with resectable colorectal liver metastases should receive peroperative chemotherapy.3 However, despite a slight increase in DFS, chemotherapy also have toxic effects, either systemic or “locoregional” (liver damage).3,6

Clinical prognostic factors of recurrence have been used to select patients to peroperative chemotherapy and to surgery, but they demonstrated a poor predictive value in terms of long-term outcome.8 In an attempt to derive more robust prognostic information, some authors have combined multiple clinical prognostic factors to formulate multiparametric scoring systems. The first scoring system for patients with colorectal liver metastases was introduced in 1996 by Nordlinger et al, subsequently followed in 1999 by Iwatsuki et al and by Fong et al.9–11 Despite promising predictive value in training data sets, all the proposed scoring systems demonstrated limited external validation and their clinical utility remains controversial.12,13

On the basis of the findings, a robust rationale emerges clearly, implicating MACC1 and possibly the downstream HGF/MET axis, in the progression of primary tumors toward metastases. MACC1 mRNA is expressed both in primary colon cancer and in colorectal liver metastases. However, to our knowledge, no studies have analyzed the prognostic impact of MACC1 mRNA expression in tissue specimens of colorectal liver metastases.

Aim of the present study is to determine the prognostic relevance of MACC1, HGF, and MET gene dosage and mRNA expression levels on recurrence-free survival in patients undergoing curative liver resection for colorectal liver metastases.

METHODS

Between October 2008 and March 2010, 113 patients underwent curative liver resection for colorectal metastases at the
Department of Surgical Oncology of the Institute for the Research and Cure of Cancer (IRCC, Candiolo, Turin, Italy) and at the Hepato-bilio-pancreatic surgical department of the Mauriziano “Umberto I” Hospital (Turin, Italy). Of these 113 patients, 64 who had complete molecular and clinical data represent the object of the present study.

Preoperative Workup and Selection Criteria for Surgery

Measurement of carcinoembryonic antigen (CEA) levels, contrast-enhanced triple-phase computed tomography (CT) of the thorax and abdomen, and magnetic resonance imaging (MRI) with mangafodipir trisodium were performed routinely for preoperative staging. 18F-fluorodeoxyglucose positron-emission tomography was used in selected patients. Response to neoadjuvant chemotherapy was assessed by CT and MRI according to response evaluation criteria in solid tumours.30

The indocyanine green retention test was routinely performed before surgery to assess liver function. Intraoperative ultrasonography was routinely performed.

Patients were considered candidates for liver resection if all liver metastases were technically resectable with curative intent.31 Presence of extrahepatic disease amenable to radical surgery was not considered a contraindication to resection.

Neoadjuvant chemotherapy was performed in patients with initially unresectable hepatic/extrahepatic metastases. All patients were periodicaly reviewed by a multidisciplinary team (hepatobiliary surgeon, oncologist, and radiologist). Liver surgery was performed as soon as metastases became technically resectable.

Adjuvant chemotherapy was not performed routinely but was based on performance status, prognostic factors, and on the number and toxicity of neoadjuvant chemotherapy courses.

Follow-up

Patients underwent abdominal ultrasonography and measurement of serum CEA levels every 4 months during the first 2 years and every 6 months thereafter. CT of the chest and abdomen was scheduled yearly or carried out whenever a recurrence was suspected. DFS was evaluated on first metastatic relapse after liver metastasis resection, thus we defined as good prognosis the patients with DFS greater than 36 months.

Analyte Extraction

Nucleic acids were isolated from surgically resected colorectal cancer liver metastases and from matched normal liver tissues, after overnight incubation of the fresh specimens in RNAlater (Ambion), followed by quick freezing at −80°C and mechanical fragmentation. Genomic DNA was isolated using the Blood and Cell Culture DNA Midi Kit (Qiagen). Total RNA was extracted using the miRNeasy Mini Kit (Qiagen) and quality checked with an Agilent 2100 Bioanalyzer (Agilent Technologies). DNA and RNA concentrations were quantified using a Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific).

Quantitative realtime polymerase chain reaction (PCR) (qPCR)-based MACC1, MET, and HGF gene copy number and mRNA expression together with mutational profile for KRAS, BRAF, PIK3CA, and NRAS were previously performed on this cohort of patients.32,33 Accordingly, the MACC1 qPCR score was obtained by subtracting the MACC1 ΔCt of a given sample from the median ΔCt of all samples (Ct values of the HPRT1 and SDHA housekeeper genes were averaged for the ΔCt calculation). Therefore, the score corresponds to the log 2 ratio between MACC1 expression in the sample and median MACC1 expression in all samples.

Statistics

All statistical analyses were performed with R-Bioconductor34: univariate and multivariate analyses were performed with the Survival package35 and ROC with ROCR.36 Significance for ROC curves was evaluated with the Wilcoxon test.

To statistically evaluate known clinical prognostic indicators in our patients set, we defined cutoff values according to the work of Fong et al.10 In particular, we considered as poor prognosis indicators the following 5 parameters: (i) initial disease stage = N+; (ii) synchronous metastasis, or metastatic recurrence within 12 months after primary resection; (iii) number of metastatic nodes greater than 1; (iv) maximum liver node diameter > 50 mm; and (v) preoperative CEA > 200 ng/mL. All analyses were censored at 3 years, so that poor prognosis cases were those with diagnosed recurrence within 36 months.

To classify patients on the basis of MACC1 expression, we calculated the fifth percentile of the MACC1 mRNA qPCR score in the group of patients who has recurrence within 3 years. Patients with MACC1 score below the threshold were classified as low risk and the remaining patients as high risk. In this way, we considered that the chosen threshold should bring an acceptable 5% of false negatives (cases with recurrence within 36 months classified as low risk). To evaluate the robustness of the threshold, we performed a Monte Carlo simulation with 10,000 iterations37 where, in each iteration, samples were randomly reassorted in good and poor prognosis groups and the threshold was chosen as the fifth percentile of the random poor prognosis group. Distribution of the 10,000 random threshold values was significantly lower than the real threshold, indicating that, overall, true poor prognosis samples have higher MACC1 expression ($P < 0.005$).

RESULTS

Perioperative Clinical and Molecular Characteristics

The clinical and molecular characteristics of the 64 patients whose tumor samples were used are described in Table 1. There were 52 men with a mean age of 66.4 years. In more than two thirds of the patients, the primary tumor was located in the colon and had regional metastatic lymph nodes. Liver metastases were diagnosed synchronously in 28 (43.8%) patients; in the 36 metachronous patients, the mean interval free of disease was 16 months (SD = 9). Most of the patients had multiple, small liver metastases. No patients had extrhepatic disease at the time of liver resection. At final pathology analysis, all the patients had a negative resection margin.

Thirty-six patients (56.3%) received neoadjuvant systemic chemotherapy before liver resection. The chemotherapy regimen was oxaliplatin-based in 15 patients and irinotecan-based in the remaining 20 patients; anti-VEGF monoclonal antibodies were added in 21 patients. Forty-three patients (67.2%) received adjuvant systemic chemotherapy, oxaliplatin-based in 20 patients and Irinotecan-based in 12; anti-VEGF monoclonal antibodies were added in 6 patients. Overall 23 patients (30.3%) underwent both neoadjuvant and adjuvant chemotherapy.

Prognostic Assessment of Known Clinical and Molecular Parameters

The median follow-up for disease-free patients was 33 months with 24% free of disease at 3 years. By univariate Cox regression analysis, we evaluated prognostic significance of known clinical parameters associated with long-term outcome.38 The analysis revealed significant association with poor prognosis for “Node Size” [hazard ratio (HR) = 2.741, 95% confidence interval (CI) = 1.27–5.914, $P < 0.05$] and “pre-resection CEA” (HR = 2.9, 95% CI = 1.296–6.489, $P < 0.001$).
The contribution of oncogenic mutations such as KRAS, PIK3CA, BRAF, and NRAS in the context of primary disease is undisputed; however, only recently their possible role has been explored in the context of metastatic disease. In our 64-sample set, KRAS mutation was found in 21 cases, and PIK3CA mutation was found in 7 cases. KRAS status showed significant association with DFS (HR = 5.966, 95% CI: 1.426–24.96, P = 0.0144) and Kaplan-Meier with log-rank test, which highlighted a 1-year longer DFS for low-MACC1 patients (median DFS = 32.63 vs 20.23 months, P < 0.01; Fig. 2A). Interestingly, the percentage of relapses was substantially lower in low-MACC1 (15.4%) versus high-MACC1 patients (64.7%; Fischer exact test, P < 0.005; Fig. 2B).

**MACC1 is an Independent Prognostic Classifier**

In the sample set analyzed, 4 prognostic parameters were statistically significant in univariate analysis: "MACC1," "KRAS," "pre-operative CEA levels," and "Node Size." To evaluate possible dependencies between these parameters, we carried out multivariate Cox regression analysis. The results, shown in Table 3 (Fig. 3), confirmed "MACC1" as an independent predictor of DFS. Interestingly, only 2 other variables ("Node Size" and "pre-operative CEA") remained significant in this analysis.

We then assessed the distribution of all variables considered for multivariate analysis within the high-MACC1 and low-MACC1 subgroups. "Node Size" and "Preop CEA" values were evenly distributed across MACC1 expression values. Interestingly, instead, KRAS mutation was found to be more frequent in high-MACC1 cases (19/51, 37.3%) than in low-MACC1 cases (2/13, 15.4%). Although not statistically significant (Fisher exact test, P = 0.1912), this correlation between KRAS mutation and high MACC1 may explain the loss of prognostic significance for KRAS mutation in multivariate analysis.

Finally, we tentatively stratified patients taking into account the 3 parameters found to be independently significant in multivariate analysis: "Node Size," "Preop CEA," and MACC1. A score was calculated as cumulative recurrence risk index (RRI), ranging therefore from 0, for patients with no positives (lowest risk), to 3, for patients positive to all parameters. Interestingly, as shown in Figure 4, cases with an index of zero had an extremely low recurrence risk, whereas cases with an index of three had a very high recurrence risk.
FIGURE 1. ROC curve analysis for (A) MACC1, (B) MET, and (C) HGF mRNA levels

7 of 10 cases with index = 2 had recurrence within 1 year. None of the patients had a score of 3.

DISCUSSION

MACC1 has been originally identified through genome-wide expression analyses, comparing primary and metastatic colon cancers. On the basis of these, MACC1 over expression was proposed as an independent prognostic indicator of metastatic dissemination, which correlates with enhanced invasion and aggressiveness of the primary tumors. MACC1 is known to promote transcription of the MET gene, thereby activating the HGF/MET axis and promoting tumor cell motility and invasion; recently a more complex regulatory network involving downregulation of miRNAs targeting both MACC1—namely miR-143—and MET—namely miR-1—has been implicated in the promotion of colon cancer cell invasion.

However, we previously observed that pharmacological blockade of MET does not abrogate in vivo growth of metastatic colorectal cancer, whereas in vivo blockade of MACC1 was found to inhibit metastatic dissemination in mouse models, suggesting that MACC1 may operate through additional pathways and mechanisms. On
nificantly higher rates of recurrence within 36 months. The impact of MACC1 expression in metastatic colorectal cancer after curative liver resection: high MACC1 levels are associated with significantly higher rates of recurrence within 36 months. A potential explanation for this apparent paradox is threefold: (i) the increased invasive potential due to MACC1 overexpression could underlie preexistent and diffused patterns of undetectable micrometastatic dissemination of the primary lesion, so that surgery of liver metastases only apparently eradicates the disease; (ii) the metastases overexpressing MACC1 could be locally more invasive per se, thus increasing the risk of secondary micrometastasization before resection; (iii) beside its proinvasive properties, MACC1 could also elicit additional effects on growth or survival of cancer cells. Indeed, we and others have proposed that further MACC1-activity mediators could be hypothesized beside MET.32,40 MACC1 expression is endowed with a stronger prognostic power than MET mRNA, which would argue against the idea that MET is the sole mediator of MACC1 biological effects, in line with previous findings.17,19,27 Further studies are needed to clarify this issue, which will involve both high-throughput expression analyses and functional validation experiments to identify and test the biological relevance of other, MET-independent MACC1 targets within the context of colorectal cancer progression.

The potential clinical implications of MACC1 as independent prognostic factor of recurrence are based on its ability to identify 2 classes of recurrence risk among the patients undergone curative liver resection for colorectal metastases. In Europe, perioperative chemotherapy is a common approach even for patients with resectable colorectal metastases. Recently, a panel of experts has recommended that most patients with resectable colorectal liver metastases should receive perioperative chemotherapy.4 Particularly, systemic chemotherapy after liver resection of colorectal metastasis is offered to all the patients fit to treatment. However, a pooled analysis of adjuvant studies has showed only nonsignificant 10% increase in DFS with a grade 3 to 4 toxicity in about 30% of the treated patients.6 Overall, the benefit of adjuvant treatment seems extremely limited, which calls for refining its use to high-risk patients' subpopulations, thus preventing useless overtreatment and associated toxicities. As an additional consequence, significant treatment-associated expenses could be spared, which is as a whole would improve cost-effectiveness of the therapeutic approach.30 In the present study, low-MACC1, good prognosis patients had a significantly lower recurrence rate (2 of 13; 15.4%) than high-MACC1, poor prognosis patients (35 of 51; 68.6%; \( P = 0.01 \)). Moreover, the prognostic power of MACC1 expression seems to be unrelated to adjuvant therapy (HR = 6.5 in patients treated with adjuvant therapy, HR = 4.3 for patients not treated) so that a relevant contribution of MACC1 levels in predicting treatment efficacy is unlikely.

This reinforces the notion that MACC1 is a pure prognostic indicator that could be exploited to inform rational therapeutic decisions after surgical intervention. If validated in larger cohorts of patients, our results could justify a MACC1-based categorization of patients, to spare good prognosis patients from useless adjuvant treatment. The technique used to measure MACC1 mRNA levels, that is, quantitative realtime PCR, is a well-established procedure that has successfully
been employed for prognostic assessment in various types of cancer, including colorectal cancer, which facilitates further assessments and extensive clinical validation. Moreover, the strong independence of MACC1 from other clinical parameters of prognostic value, that is, “Node Size” and “Preop-CEA,” holds promise for further integration and risk stratification scores.

Interestingly, the negative prognostic impact of KRAS mutation on liver metastatic colorectal cancer is confirmed in our data. However, its prognostic significance is lost in multivariate analysis, when MACC1 is included. This finding, together with the fact that KRAS mutation is more frequent in high-MACC1 cases, highlights possible cooperation between the 2 oncogenes and, if further validated in larger cohorts of patients, could increase the interest for MACC1 assessment in clinical practice.

**CONCLUSIONS**

If confirmed, the results presented here could pave the way for the inclusion of MACC1 expression analysis in a multiparametric score (including molecular, clinical and pathological features) that could help the clinician in assigning aggressive, mild, or even non-adjuvant regimens to resected patients, depending on their relapse risk, even in the context of metastatic diseases. We are planning to validate both the prognostic relevance of MACC1 and its integration with clinical prognostic factors in a larger cohort of patients undergoing curative liver resection of colorectal cancer metastases.

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