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mRNA expression profiles of colorectal liver metastases as a novel biomarker for early recurrence after partial hepatectomy

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ABSTRACT

Background: Identification of specific risk groups for recurrence after surgery for isolated colorectal liver metastases (CRLM) remains challenging due to the heterogeneity of the disease. Classical clinicopathologic parameters have limited prognostic value. The aim of this study was to identify a gene expression signature measured in CRLM discriminating early from late recurrence after partial hepatectomy.

Methods: CRLM from two patient groups were collected: I) with recurrent disease ≤12 months after surgery (N = 33), and II) without recurrences and disease free for ≥36 months (N = 30). The patients were clinically homogeneous; all had a low clinical risk score (0–2) and did not receive (neo-) adjuvant chemotherapy. Total RNA was hybridised to Illumina arrays, and processed for analysis. A leave-one-out cross validation (LOOCV) analysis was performed to identify a prognostic gene expression signature.

Results: LOOCV yielded an 11-gene profile with prognostic value in relation to recurrent disease ≤12 months after partial hepatectomy. This signature had a sensitivity of 81.8%, with a specificity of 66.7% for predicting recurrences (≤12 months) versus no recurrences for at least 36 months after surgery (X^2 $P < 0.0001$).

Conclusion: The current study yielded an 11-gene signature at mRNA level in CRLM discriminating early from late or no relapse after partial hepatectomy.

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1. Introduction

Q1 Colorectal cancer is one of the most commonly diagnosed cancers worldwide (Torre et al., 2015). Approximately 15–25% of patients with colorectal cancer (CRC) present with synchronous liver metastases and another 20% have a metachronous disease development (van der Pool et al., 2012). For patients presenting with isolated liver metastases, partial hepatectomy is the only potentially curative treatment option. Reported 5-year survival rates are 40–60% (Dols et al., 2009; Rees et al., 2008; Primrose, 2010). A substantial number of patients develop recurrent disease after liver surgery, underlining the need for prognostic biomarkers (D'Angelica et al., 2011; Butte et al., 2015; de Jong et al., 2009). Such prognostic biomarkers may allow a more personalised treatment strategy. In recent years, several clinicopathological prognostic variables in patients with isolated colorectal liver metastases (CRLM) have been identified predicting the risk of relapse after a metastasectomy (Matias et al., 2015). These variables have been integrated in various clinical risk scores (CRS) (Matias et al., 2015; Fong et al., 1999; Konopke et al., 2009; Nagashima et al., 2004; Nordlinger et al., 1996). The CRS according to Fong et al. is the most widely used and validated score, able to distinguish between high risk and low risk patients in terms of survival outcomes (Fong et al., 1999). This score is composed of 5 prognostic variables: positive lymph node status of the primary tumour, diagnosis of liver metastases within 12 months after resection of primary tumour, serum CEA ≥200 ng/ml, >1 liver metastases, a metastasis of >5 cm diameter. Each variable accounts for 1 point. Patients with 0–2 points are categorised as low risk, patients with 3–5 points as high risk. Still, outcomes after surgery remain heterogeneous: low risk patients may develop early recurrences – approximately 50% of patients with a low CRS develop metastases within 12 months after surgery – while high risk patients may remain disease free (Poston, 2008; Poston et al., 2008). Unravelling the biological properties characterising tumours may be pivotal to designing individualised therapies, based on biological predictors of outcome rather than or in addition to clinical predictors. Various groups have established molecular subtypes in primary cancers with distinct biology, predictive and prognostic value (Guinney et al., 2015;Paik et al., 2004; Hoshida et al., 2008; Albain et al., 2010; Budinska et al., 2013; Sadanandam et al., 2013). Biological markers may improve patient selection for (neo-) adjuvant therapies in addition to surgical management or intensive surveillance schemes.

The ability to analyse tumours at DNA-, RNA-, and protein-level promises to revolutionize our understanding of the malignant disease process, and hopefully this will herald new (superior) biomarkers. The aim of the current study was to identify a prognostic gene signature at mRNA level in patients with a low CRS, effective in identifying patients at high risk of early recurrence after surgery for CRLM.

1.1. Methods

1.1.1. Patients and treatment

Erasmus MC Cancer Institute is a tertiary referral centre for liver surgery. In the current retrospective study, patient characteristics were collected from a prospectively maintained database. All patients undergoing resection for CRLM are prospectively entered into an institutional database. This database includes standard clinicopathological variables. Patients selected for the current study had a low risk profile (Fong's clinical risk score 0–2 (Fong et al., 1999)) and did not receive treatment with (neo-) adjuvant chemotherapy for the resectable CRLM in line with the Dutch guidelines that do not support routine administration of chemotherapy/biologicals in the case of primary resectable colorectal liver-only metastases. Patients were further selected according to the following criteria: I) patients with recurrent disease within 12 months after hepatectomy, and II) patients without recurrent disease and a disease free survival of at least 36 months after hepatectomy. Thus, "two extremes" were selected in terms of recurrent disease. All resections were performed between 2000 and 2009. Hepatic parenchymal resection was performed with an ultrasonic surgical aspirator and a monopolar coagulator. R0-resections were defined by the absence of microscopic tumour invasion of the resection margins, and R1-resections were defined by the presence or microscopic tumour invasion of the resection margins (Ayez et al., 2012).

During follow-up, patients visited the outpatient clinic every 4 months in the first 2 years after CRLM resection for clinical examination and CEA-determination. Thereafter, patients visited the outpatient clinic every 6 months and were discharged from follow up after 5 years. Abdominal imaging (CT of thorax and abdomen) was performed twice a year during the first 3 years and thereafter annually. If disease recurred, a decision on whether to initiate chemotherapy treatment or to perform local therapy was made by a multidisciplinary team. Disease free survival (DFS) was defined as the interval in months between resection of CRLM and recurrence.

1.1.2. Tissue collection and assessment

After resection of CRLM, tumour tissue is standardly fixed on formalin and embedded in paraffin in the department of pathology according to standard protocols, and stored. For the current study, tumour samples (N = 80) of CRLM were retrieved from the selected patient groups. In the case a patient had more than one metastasis, there were no additional selection criteria in terms of which tumour to analyse. The formalin fixed, paraffin embedded (FFPE) samples were evaluated by a pathologist for colon tumour cell content: only specimens with at least 30% tumour cells in the tissue block were included (N = 63). The final study population consisted of 33 samples for group I with disease recurrence within 12 months and 30 samples for group II without disease recurrence and a DFS of 36 months.

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