Clin Exp Metastasis (2013) 30:457–470
DOI 10.1007/s10585-012-9551-8

RESEARCH PAPER

# The tumour biology of synchronous and metachronous colorectal liver metastases: a systematic review

**A. A. P. Slesser · P. Georgiou · G. Brown · S. Mudan · R. Goldin · P. Tekkis**

Received: 11 August 2012 / Accepted: 9 November 2012 / Published online: 23 November 2012


**Abstract** Forty to fifty percent of colorectal cancer (CRC) patients develop colorectal liver metastases (CLM) that are either synchronous or metachronous in presentation. Clarifying whether there is a biological difference between the two groups of liver metastases or their primaries could have important clinical implications. A systematic review was performed using the following resources: MEDLINE from PubMed (1950 to present), Embase, Cochrane and the Web of Knowledge. Thirty-one articles met the inclusion criteria. The review demonstrated that the majority of studies found differences in molecular marker expression between colorectal liver metastases and their respective primaries in both the synchronous and metachronous groups. Studies investigating genetic aberrations demonstrated that the majority of changes in the primary tumour were 'maintained' in the colorectal liver metastases. A limited number of studies compared the primary tumours of the synchronous and metachronous groups and generally demonstrated no differences in marker expression. Although there were conflicting results, the colorectal liver metastases in the synchronous and metachronous groups demonstrated some differences in keeping with a more aggressive tumour subtype in the synchronous group. This review suggests that biological differences may exist between the liver metastases of the synchronous and metachronous groups. Whether there are biological differences between the primaries of the synchronous and metachronous groups remains undetermined due to the limited number of studies available. Future research is required to determine whether differences exist between the two groups and should include comparisons of the primary tumours.

**Keywords** Colorectal cancer · Colorectal liver metastases · Molecular markers · Biomarkers · Synchronous colorectal liver metastases · Metachronous colorectal liver metastases

**Abbreviations**

| | |
|---|---|
| CLM | Colorectal liver metastases |
| CRC | Colorectal cancer |
| SCLM | Synchronous colorectal liver metastases |
| IHC | Immunohistochemistry |
| RT-PCR | Reverse transcription polymerase chain reaction |
| 2D-DIGE | Two-dimensional difference gel electrophoresis |
| FISH | Fluorescence in situ hybridization |
| CEBM | Oxford Centre for Evidence-Based Medicine Levels of Evidence |

A. A. P. Slesser · P. Georgiou · P. Tekkis
Department of Colorectal Surgery, The Royal Marsden Hospital, Fulham Road, London, UK
e-mail: alistair.slesser08@imperial.ac.uk

P. Georgiou · S. Mudan · P. Tekkis
Division of Surgery, Imperial College, Chelsea and Westminster Campus, London, UK

G. Brown
Department of Radiology, Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, UK

S. Mudan
Department of Hepatobiliary Surgery, Royal Marsden Hospital, Fulham Road, London SW3 6JJ, UK

R. Goldin (✉)
Centre for Pathology, Imperial College at St Mary's Hospital, Praed Street, London W2 1NY, UK
e-mail: r.goldin@imperial.ac.uk

## Introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer death worldwide [1]. The presence of colorectal liver metastases (CLM) are associated with a poor prognosis, with a median survival for untreated disease ranging between six and twelve months [2–4]. Surgical intervention is the only chance of long-term survival, with the five year survival ranging between 25 and 58 % [4–6]. Unfortunately, forty to fifty per cent of CRC patients develop CLM [6, 7]. They are either synchronous or metachronous in presentation with approximately equal incidence [4, 6, 7]. There is no clear international definition of what constitutes a synchronous presentation, the 7th edition of the AJCC manual states that staging can be undertaken as part of 'definitive surgery, as part of primary treatment or within 4 months of diagnosis, whichever is longer' [8]. However, no consensus exists in the literature with varied interpretations being used in clinical studies including: metastases detected prior to or at the time of surgery, metastases detected within three or twelve months of the CRC diagnosis [5, 9]. Patients who present with synchronous colorectal liver metastases have locally advanced primary tumours and tend to present with a greater metastatic burden than patients who develop metachronous colorectal liver metastases [10, 11]. It has been demonstrated that the presence of synchronous disease is an indicator of poor prognosis [10]. There is no consensus as to why colorectal cancer primaries develop either synchronous or metachronous CLM [5]. Clarifying whether synchronous and metachronous CLM represent different subtypes of metastatic CRC is paramount as it could have important clinical implications. The aim of this study was to ascertain whether there was a biological difference between the two subsets of patients. The reader is advised to refer to Table 1 for a brief description of the markers discussed in this review.

## Methods

A systematic review of the literature was performed to assess the differences in biomarker expression: (1) between patients with synchronous colorectal liver metastases (synchronous group) and patients with metachronous colorectal liver metastases (metachronous group) and (2)to assess differences in biomarker expression between colorectal liver metastases and their respective CRC primaries in both the synchronous and metachronous groups (Fig. 1). The methodology undertaken was based on the guidelines from the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement [12].

### Search strategy

An electronic database search was performed in October 2012 using the following resources: MEDLINE from PubMed (1950 to present), Embase, Cochrane and the web of knowledge. The following search headings were used: "colorectal cancer liver metastases", "colorectal cancer hepatic metastases", "colorectal cancer synchronous liver metastases", "colorectal cancer synchronous hepatic metastases", "colorectal cancer metachronous liver metastases", "colorectal cancer metachronous hepatic metastases" combined with the Boolean operator 'AND' and each of the following terms: "biomarkers" and "molecular markers". The titles were initially scanned and abstracts of interest were reviewed. All articles reviewed and included in the study had their reference lists scanned and studies found were included in the study if they met the inclusion criteria.

### Inclusion criteria

To enter the review the study had to:

(1) compare biomarkers between the defined subgroups (Fig. 1); (2) only include studies assessing liver metastases or primary tumours of colorectal adenocarcinoma origin; (3)differentiate between colorectal liver metastases and extra-hepatic metastases during the tissue analysis.

### Exclusion criteria

The following criteria were used to exclude studies from the review:

(1) studies that analysed synchronous and metachronous groups together as one entity; (2) studies that analysed CLM with other types of metastases as one entity; (3) studies that did not clearly define whether CLM were synchronous or metachronous in origin; (4)animal studies; (5) articles that were conference abstracts, editorials, commentaries/letters or reviews.

### Data extraction

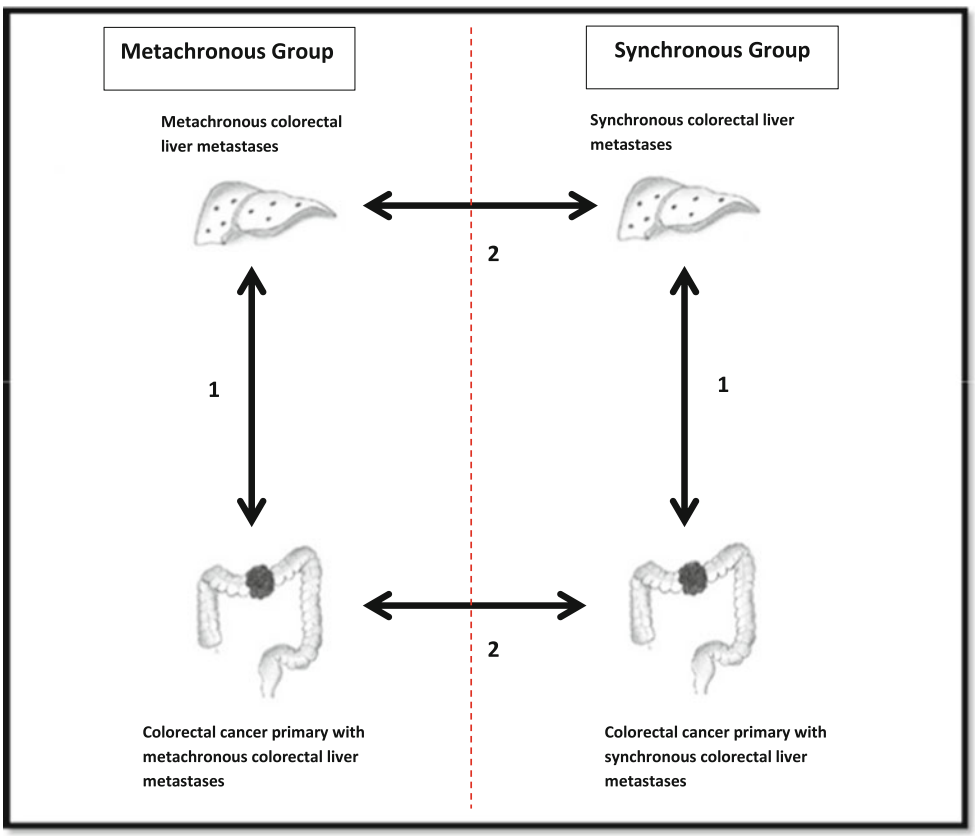
Two authors (AS, PG) independently collected and tabulated the data into an electronic spread-sheet. Any differences in collated data between the two authors were discussed and agreement was reached by consensus. The specific data items collected were the following: first author/institute, year of publication, year of study, study design, groups being compared, study sample size, molecular markers being assessed, manner of molecular marker assessment, and significant/non-significant findings. The study quality was assessed by two authors independently using the Newcastle-Ottawa Scale for assessing the quality of non-randomised trials.

**Table 1** Description of molecular markers assessed in the systematic review

| Molecular marker | Abbreviation | Description | Role in colorectal cancer |
|---|---|---|---|
| Angiopoietins | Ang-1/2 | Tie2 receptor ligands involved in angiogenesis | Ang-2 promotes angiogenesis. Ang-1 promotes vascular maturation |
| Amphiregulin | AREG | Ligand epidermal growth factor receptor | Thought to be involved in development of liver metastases |
| Chemokine receptor 6 | CCR6 | Chemokine receptor for ligand CCL20 | Expressed in CRC. Thought to be involved in CLM development |
| Cluster of differentiation 34 | CD34 | Marker of endothelial cells and microvessel density | Microvessel density correlates with CRC stage and metastatses |
| Cluster of differentiation 83 | CD83 | Marker of mature dendritic cells | Correlates with stage of CRC and presence of distant metastases |
| Cyclin-dependent kinases 2 | CDK2 | Involved in cell cycle regulation | Thought to be involved in CRC progression |
| Carcinoembryonic antigen | CEA | Soluble glycoprotein | Involved in the development of liver metastases |
| c-erbB-2 | – | Glycoprotein receptor tyrosine kinase | Involved in cellular proliferation in CRC |
| Chromosomes 8,18,14,22,20 | – | – | 8, 14, 18 and 22 involved in carcinogenesis. 20 associated with CLM |
| Cyclo-oxygenase-2 | COX-2 | Converts arachidonic acid to prostaglandin-$H_2$ | Correlates with CRC progression and the presence of CLM |
| Chemokine receptor type 4 | CXCR4 | Chemokine receptor for ligand CXCL12 | Correlates with advanced CRC and has role in metastatic development |
| Cyclin E | – | Involved in cell cycle regulation | Involved in cell proliferation and carcinogenesis |
| Dihydropyrimidine dehydrogenase | DPD | Enzyme involved in pyrimidine catabolism | Involved in the catabolism of 5-FU based agents |
| Epidermal growth factor recptor | EGFR | Tyrosine kinase | Important role in the progression and metastatic potential of CRC |
| Excision repair cross-complementing factor1 | ERCC1 | Nucleotide excision repair | Confers ability to repair platinum related DNA damage |
| Epiregulin | EREG | Ligand for epidermal growth factor receptor | Involved in development of liver metastases |
| Focal adhesion kinase | FAK | Tyrosine kinase involved in cell migration | Thought to have a role in the metastatic development |
| Insulin receptor substrate 1 | IRS1 | Insulin receptor substrate | Involved in CRC progression via the β-catenin signalling pathway |
| Ki-67 | – | Marker of cell proliferation | Unspecific to stage of CRC |
| Methylated in tumour 1 | MINT1 | CpG sequence known to be methylated in tumours | Demonstrated to be methylated in CRC |
| Matrix metalloproteinases | MMP | Proteolytic enzymes | Important role in progression and development of metastases |
| Orotate phosphoribosyl transferase | OPRT | Enzyme involved in the synthesis of pyrimidine nucleotides | Enzyme involved in activating 5-FU |
| p27 | – | Cyclin-dependent kinase inhibitor regulating cell cycle | Reduced expression correlates with advanced stages of CRC |
| p53 | – | Inducer of apoptosis | Mutations of p53 occur in about 50 % of sporadic CRCs |
| Paxillin, Vinculin, Talin | – | Proteins associated with the focal-adhesion-complex | Correlate with carcinogenesis and metastasis in CRC |
| Placenta growth factor | PLGF | Angiogenic factor | Associated with CRC progression |
| Sialyl Lewis A | Sialyl Le[a] | Family of carbohydrate ligands found on CEA | Thought to be involved in CRC progression |
| Transforming growth factor-α | TGF-α | Growth factor and member of the epidermal growth factor family | Involved in CRC progression |
| Tissue inhibitor of metalloproteinases | TIMP | Family of endogenous inhibitors of MMPs | Demonstrated to reduce growth of CRCs |
| Thymidine phosphorylase | TP | Enzyme of the pyrimidine salvage pathway | Enzyme involved in the activation of 5-FU chemotherapy |

**Table 1** continued

| Molecular marker | Abbreviation | Description | Role in colorectal cancer |
|---|---|---|---|
| Thymidylate synthase | TS | Enzyme involved in the synthesis of thymidine monophosphate | Target of 5-fluorouracil chemotherapy |
| Uridine phosphorylase | UP | Enzyme that converts uridine to uracil | Involved in the activation of 5-FU chemotherapy |
| Vascular Endothelial Growth Factor | VEGF | Angiogenic factor | Important role in the progression of CRC and development metastases |
| Vascular endothelial growth factor receptor | VEGFR | Tyrosine kinase receptor for VEGF | Expression associated with CRC progression and poor prognosis |
| Zinc finger E-box binding homeobox-2 | ZEB2 | Transcription factor involved in epithelial mesenchymal transition | Associated with CRC progression and development of metastases |




*Arrows demonstrate subgroups compared;* ***1****: Colorectal liver metastases compared to their respective colorectal cancer primaries in either the Metachronous or Synchronous groups;* ***2****: Comparison of the colorectal cancer primaries and colorectal liver metastases between the Metachronous and Synchronous groups.*

**Fig. 1** Diagram demonstrating the different groups in which molecular markers were compared

### Data analysis/quality of studies

All studies were assessed for their level of evidence using the Oxford Centre for Evidence-Based Medicine Levels of Evidence table [13]. It was elected to perform a descriptive review of the data as opposed to a meta-analysis due to the heterogeneity of the studies and markers assessed.

## Results

### Study selection and quality

A total of 3400 citations including references were found. After exclusion of duplicate citations, a review of the titles and abstracts resulted in 213 articles being reviewed of

which 31 met the inclusion criteria (Fig. 2). The overall quality of the studies was poor with a median Newcastle-Ottawa score of four (1-6).

## Study design and characteristics

All of the studies were retrospective and the publication year of the articles ranged between 1996 and 2012, with the majority (25) being published in or after the year 2001. None of the studies were randomised and were all observational. Fourteen of the studies had been undertaken in Japan.

## Comparison of molecular markers between colorectal cancer liver metastases and colorectal cancer primaries in the synchronous and metachronous groups

There were a total of fifteen studies assessing the difference in molecular marker expression between the colorectal liver metastases and their associated CRC primaries (Table 2). In the metachronous group, the expression of TS, ERCC1, DPD was found to be higher in the liver metastases. The expression of p27 using immunohistochemistry (IHC) was significantly reduced in the liver metastases of the metachronous group. No significant differences in p27 mRNA, p53, Ki-67, AREG, EREG were found in the metachronous group.

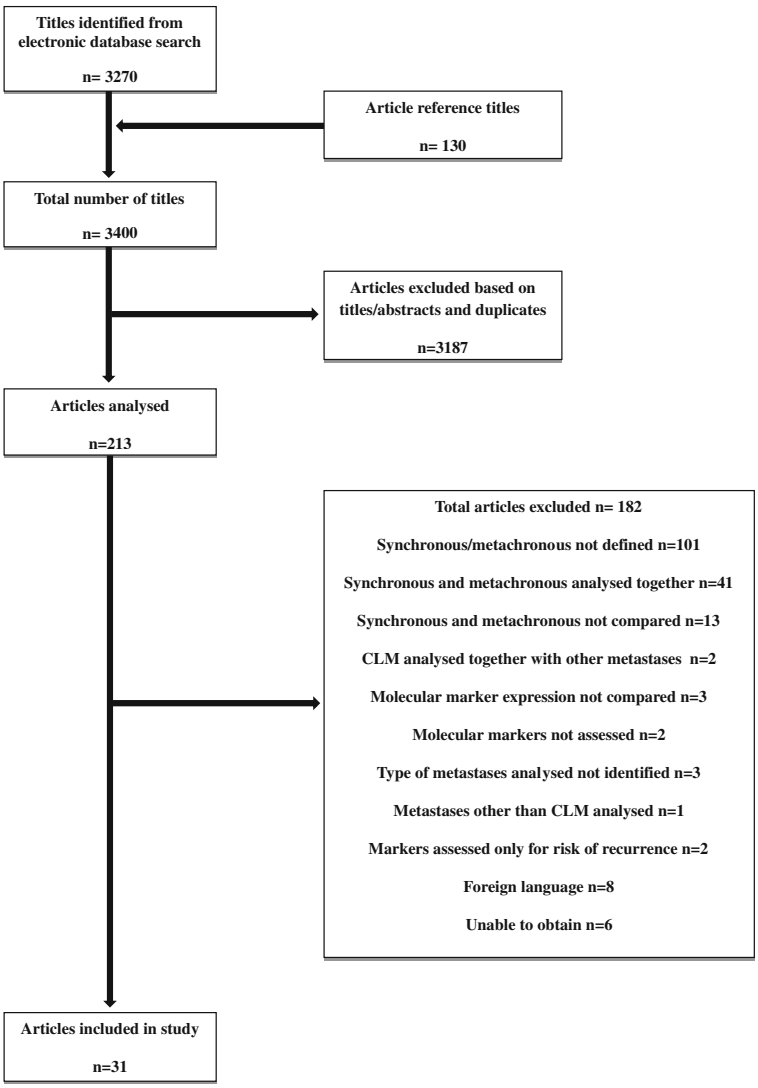



**Fig. 2** Flowchart demonstrating the search strategy

**Table 2** Comparison of molecular marker expression in colorectal liver metastases and associated primaries

| Author | Years | Cases | Definition of synchronous/ metachronous | Molecular markers assessed | Method of analysis | Controls | Molecular markers findings in metachronous group | Molecular markers findings in synchronous group | CEBM |
|---|---|---|---|---|---|---|---|---|---|
| Kobayashi et al. [21] | 2008 | 31 | None | ERCC1 and TS mRNA | RT-PCR | No | TS and ERCC1 mRNA levels were higher in liver metastases $p = 0.0084, 0.037$. TS mRNA in primaries significantly correlated with levels in matched liver metastases | ERCC1 mRNA primary significantly correlated with levels in CLM $p = 0.0038$.<br>TS mRNA in primaries significantly correlated with levels in matched liver metastases.<br>No difference in TS and ERCC1 mRNA levels $p = 0.65, 0.20$. | 4 |
| Thomas et al. [18] | 1998 | 36 | Synchronous- detected at primary resection.<br>Metachronous- $> 6$ months | p27, p53, Ki-67 | IHC, In Situ Hybridization | Yes | No significant difference in p27 mRNA, p53 or Ki-67 $p = 0.75, 1.00$.<br>Significant reduction of p27 on IHC in CLM $p = 0.03$ | No difference in p27, p53 or Ki-67 | 4 |
| Shirota et al. [43] | 2002 | 8 | None | DPD mRNA | RT-PCR | Yes | DPD mRNA had a significant higher expression in CLM p 0.017 | | 3b |
| Kuramochi et al. [31] | 2012 | 120 | None | AREG/EREG | RT-PCR | No | No significant difference in AREG or EREG mRNA levels between primary and CLM. AREG and EREG primary tumour expression levels correlated with CLM Rs 0.6, 0.36 | No significant difference in AREG or EREG mRNA levels between primary and CLM. AREG and EREG primary tumour expression levels correlated with CLM Rs 0.357, 0.58. | 3b |
| Shi et al. [44] | 2011 | 18 | None | Protein expression profile | 2D-DIGE, Western blotting, IHC | Yes | | No significant difference in protein expression profile. | 3b |
| Ghadjar et al. [23] | 2006 | 16 | None | CCR6 | IHC | Yes | | CCR6 was significantly lower in the CLM $p = 0.02$ | 3b |
| D'Arrigo et al. [45] | 2005 | 10 | None | Genetic profile | RT-PCR, cDNA arrays | Yes | | Differing expression in 6 genes out of 773 genes. | 3b |
| Goldstein et al. [46] | 2001 | 102 | Synchronous- $< 1$ month post-operative | EGFR | IHC | Yes | | No significant difference in EGFR expression. | 4 |
| Al-Mulla et al. [47] | 1999 | 7 | Synchronous- detected at time of primary surgery | Genetic profiling | DNA hybridization | Yes | | Similar chromosomal changes were 'frequently' found. New genetic aberrations compared to the matched primary were found in all the CLM. | 3b |
| Kim et al. [24] | 2005 | 14 | None | CXCR4 expression | RT-PCR | Yes | | CXCR4 mRNA expression significantly higher in CLM $p = 0.0005$ | 3b |

**Table 2** continued

| Author | Years | Cases | Definition of synchronous/ metachronous | Molecular markers assessed | Method of analysis | Controls | Molecular markers findings in metachronous group | Molecular markers findings in synchronous group | CEBM |
|---|---|---|---|---|---|---|---|---|---|
| Ayaki et al. [30] | 2001 | 10 | None | FAK, Paxillin, Vinculin and Talin | IHC, Western Blot Analysis | Yes | | FAK expression significantly lower in CLM $p = 0.022$.<br>No significant diference in Vinculin, Paxillin, Talin expression. | 3b |
| Takahashi et al. [48] | 2006 | 9 | None | Genetic expression profile | DNA micorarray, FISH | Yes | | No difference in gene expression. | 3b |
| Kim et al. [27] | 2010 | 10 | None | CEA, VEGF, MMP-1, MMP-7 and TIMP-1 | IHC | Yes | | Higher VEGF expression in CLM $p = 0.009$.<br>No difference in expression of MMP-1, MMP-2, CEA or TIMP-1. | 3b |
| Li et al. [20] | 2001 | 10 | None | Cyclin E, CDK2, Ki67 | IHC | Yes | | Cyclin E expression decreased significantly in CLM compared to matched primaries $p = 0.0469$. No difference in expression of CDK2 or Ki67 $p = 0.1975$, 0.1813 | 3b |
| Esposito et al. [28] | 2012 | 24 | None | IRS1 | IHC | Yes | | Significantly higher expression of IRS1 in CLM compared to primaries $p < 0.01$. | 3b |

In the synchronous group, the expression of VEGF, CXCR4 and ISR1 were found to be significantly higher in the liver metastases. The expression of Cyclin E, CCR6 and FAK were lower in the liver metastases of the synchronous group. No significant differences in genetic aberration, protein expression profile or expression of ERCC1, TS, p27, p53, Ki-67, AREG, EREG and EGFR were found in the synchronous group.

### Comparison of molecular markers in synchronous group compared to metachronous group

A total of 16 studies compared the tumour marker expression between the two groups (Table 3). In the synchronous group, the CRC primaries were found to have a significantly higher methylation level of MINT1 than the primaries of the metachronous group. No difference in the expression of TGF-α, EGFR, Ki-67, p53, VEGF, CEA, sialyl LeA was found between the primaries of the two groups. The liver metastases in the synchronous group were found to have an increased expression of COX-2 mRNA, TGF-α and a higher Angiopoietin-2/Angiopoietin-1 ratio compared to the liver metastases in the metachronous group. The expression of CD83 and EGFR mRNA were found to be higher in the liver metastases of the metachronous group compared to the liver metastases of the synchronous group. No significant difference in the expression of COX-2 or EGFR was found between the liver metastases of the two groups using IHC. No significant differences between the metastases of the two groups were found in the following markers: VEGF, angiopoietins, genetic aberrations, Ki-67, TP, CD31, CD34, c-erb-2 and ZEB2.

## Discussion

Our review highlighted that the majority of the studies were rarely validated by further studies and generally used semi-quantative methods to analyse expression levels (Tables 2, 3). Furthermore, as other authors have found, very few of the included studies defined the time interval employed in their definitions of what constituted either a 'metachronous' or 'synchronous' presentation and it thus makes interpretation of the comparative findings difficult (Tables 2, 3) [5, 14]. Furthermore, the overall quality of the studies included was poor: nine studies did not use any controls, only 11 studies achieved a Newcastle-Ottawa score of five or more and small sample sizes were frequently used.

### Comparison of molecular marker expression between colorectal liver metastases and their respective colorectal cancer primaries

The process of the development of colorectal cancer liver metastases is still not fully understood and is complex [15, 16]. It is believed that a 'subpopulation' of cancer cells within the primary tumour evolve and develop the ability to metastasise [4]. It is recognised that CLM can exhibit biological differences to their matched primaries due to the micro-environment of the liver and the necessary genetic alterations required for the CRC tumour cells to survive the different steps of metastatic development [4, 17].

p27 has been demonstrated to correlate with advanced stages of CRC. [18, 19] In the metachronous group, its expression was reduced in the liver metastases suggesting that there was a 'post-translational' degradation of the protein in the liver metastases. Currently little is known about the role of Cyclin E in the development of CLM [20]. The expression of cyclin E was found to be significantly reduced in the liver metastases of the synchronous group [20]. It is postulated that this finding is as a result of the liver microenvironment resulting in a reduced rate of proliferation of CLM compared to the primaries [20].

Thymidylate synthase (TS) is the target of 5-fluorouracil (5-FU) and excision repair cross-complementing factor 1 (ERCC1) confers the ability to repair platinum related DNA damage [21, 22]. Kobayashi H et al. [21], demonstrated that there was no quantitative difference in expression of TS and ERCC1 expression between the CLM and their respective primaries in the synchronous group. Conversely, the expression levels of both TS and ERCC1 were significantly higher in the metastases of the metachronous group compared to their matched CRC primaries [21]. This finding would suggest that there is a difference in biology between the CLM of the synchronous and metachronous groups [21].

The expression of chemokine receptors in CRC cells has led to the belief that they play an important role in the development of CRC metastases [16, 23, 24]. An interesting finding of the review, was that the expression level of chemokine receptor CCR6 was decreased in the CLM of the synchronous group compared to their matched primaries [23]. The ligand (CCL20) for CCR6 is found predominantly in the periportal area of the liver, and it is postulated that this may be one of the mechanisms by which the CRC cells metastasize to the liver [23, 25]. The reduced expression of CCR6 found in the synchronous CLM was probably as a result of ligand binding and the subsequent degradation of the chemokine receptors [23]. CXCR4 is the most commonly expressed chemokine in CRC whilst its ligand is highly expressed in normal liver parenchyma and has been shown to have an important role in the growth of CRC liver metastases [16, 24, 26]. Indeed, this seems substantiated by the fact the expression of CXCR4 was significantly elevated in the liver metastases of the synchronous group compared to their respective CRC primaries [24]. The liver has a naturally high expression of the CXCR4 receptor ligand (CXCL12) and it

**Table 3** Expression of molecular markers in synchronous group compared to metachronous group

| Author | Years | Cases | Definition of synchronous/ metachronous | Molecular markers assessed | Method of analysis | Controls | Molecular markers findings in primaries | Molecular markers findings in colorectal liver metastases | CEBM |
|---|---|---|---|---|---|---|---|---|---|
| De Jong et al. [39] | 1998 | 45 | None | TGF-α, EGFR, Ki67 and p53 | IHC | No | No significant difference in TGF-α, EGFR, Ki67 and p53 | Higher TGF-α found in synchronous group 65 % versus 40 %. No significant difference in Ki-67 or EGFR. | 4 |
| Kuramochi et al. [49] | 2006 | 31 | None | VEGF mRNA expression | RT-PCR | Yes | No significant difference in VEGF expression $p = 0.1280$ | No significant difference in VEGF expression $p = 0.9172$ | 3b |
| Ono et al. [50] | 1996 | 44 | None | CEA and sialyl Lewis A | IHC | Yes | No significant difference in CEA or sialyl LeA expression | – | 3b |
| Nanashima et al. [51] | 1997 | 18 | None | Chromosomes 8, 18, 14/22, 20 | FISH | Yes | No significant difference in chromosome aberrations | No significant difference in chromosome aberrations. | 3b |
| Kitabatake et al. [52] | 2002 | 37 | None | Ki-67 | IHC | Yes | No significant difference in Ki-67 expression | No significant difference in Ki-67 expression.. | 3b |
| Konishi et al. [53] | 2011 | 43 | Synchronous- < 12 months after CRC diagnosis | Methylation or mutation of 13 CpG sites. | Pyrosequencing, methylated CpG island amplification | Yes | MINT1 methylation significantly higher in primaries of synchronous group $p = 0.0121$.<br>No differences between the rest of the CpG sites | – | 3b |
| Miyagawa et al. [41] | 2004 | 70 | None | CD83 | IHC, western blot | No | | Metachronous group had a significantly higher CD83 positive cell $p = 0.0313$. | 3b |
| Inokuchi et al. [22] | 2004 | 23 | None | TS, DPD, OPRT, TP and UP genes | RT-PCR | No | | Same level of expression of all five genes $p = 0.74$. | 4 |
| Petrowsky et al. [54] | 2001 | 41 | None | Ki-67 | IHC | No | | No significant difference in expression of Ki-67. | 4 |
| Pantaleo et al. [36] | 2008 | 18 | None | EGFR, COX-2 | Microarray, RT-PCR, Western Blot, ELISA | No | | EGFR was overexpressed in metachronous group $p = 0.046$.<br>COX-2 gene was over expressed in synchronous group $p = 0.012$ | 4 |

**Table 3** continued

| Author | Years | Cases | Definition of synchronous/ metachronous | Molecular markers assessed | Method of analysis | Controls | Molecular markers findings in primaries | Molecular markers findings in colorectal liver metastases | CEBM |
|---|---|---|---|---|---|---|---|---|---|
| Nakamoto et al. [35] | 2007 | 44 | None | VEGF-A, VEGF-C, COX-2, TP, CD34(MVD) | IHC | No | | No difference in expression of any of the markers $p = 0.572$, 0.136, 0.105, 0.954, 0.966. | 4 |
| Chen et al. [55] | 2010 | 44 | None | c-erbB-2, VEGF | IHC | No | | No significant difference in c-erbB-2 or VEGF $p = 0.786$, 0.837. | 4 |
| Kochhar et al. [56] | 1997 | 87 | None | Microsatellite instability and allelic imbalance | PCR | Yes | | No significant difference in allelic instability of 17p $p = 0.798$. | 4 |
| Nanashima et al. [57] | 2009 | 139 | None | CD34 | IHC | Yes | | No significant difference in CD34 $p = 0.12$ | 4 |
| Kahlert et al. [58] | 2011 | 30 | None | ZEB2 | IHC | Yes | | No significant difference in expression of ZEB2 at tumour centre or invasion front $p = 0.34$, 0.16 | 3b |
| Van der Wal et al. [42] | 2012 | 29 | Synchronous- detected at time of primary resection, Late synchronous- 3 and 12 months post diagnosis, Metachronous > 12 months | CD31, HIF-1α, VEGF-A, VEGFR-1/2, PLGF, Angiopoietins | RT-PCR, IHC | Yes | | No significant difference in CD31, VEGF-A, VEGFR-1/2, HIF-1α and Angiopoietins. VEGF-A, VEGFR-1, PLGF were significantly higher in the adjacent liver parenchyma of the early synchronous group compared to the adjacent liver parenchyma of the metachronous group $p = <0.05$. Significantly higher Angiopoietin-2/ Angiopoietin-1 ratio in early synchronous metastases and adjacent parenchyma compared to the metastases and adjacent parenchyma in the late synchronous and metachronous groups $p = <0.05$. | 3b |

is thought CRC cells with an increased expression of CXCR4 may have an increased ability to metastasise to the liver by a 'homing' mechanism [24].

Vascular endothelial growth factor (VEGF) has been shown to correlate with advanced CRC, lymphatic invasion and metastases [26]. Kim et al. [27], demonstrated, using immunohistochemistry, that the expression of VEGF was significantly increased in the CLM of the synchronous group compared to their CRC primaries. The increased expression of VEGF maybe as a result of a type two error or it may suggest that VEGF plays an important role in the progression of synchronous CLM and perhaps relates to the aggressive nature of synchronous CLM compared to metachronous CLM. Insulin Receptor Substrate 1 (Irs1), is thought to be involved in the β-catenin signalling pathway and is considered to have a role in CRC progression [28, 29]. The CLM in the synchronous group had a significantly higher expression of Irs1 suggesting a possible role in the development of metastases [28]. Focal adhesion kinase (FAK) is thought to play a role in metastatic adenocarcinomas [16, 30]. The expression of FAK was lower in the liver metastases of the synchronous group compared to their CRC primaries [30]. Although a small sample size of ten patients was assessed, it may denote that a reduced motility of the metastatic cells confers an advantage once the metastatic CRC cells are established in the liver.

Several studies did not demonstrate any difference in expression between the CLM and their respective primaries. In a recent study, the expression of EGFR ligands in the primary tumours correlated with their respective liver metastases in both the metachronous and synchronous groups (Table 1) [31]. In addition, studies assessing genetic aberrations and protein expression profile in the synchronous group did not demonstrate any significant differences between the primaries and their respective CLM (Table 2). This would suggest that some of changes required for metastatic progression occur at a primary level and are maintained at a metastatic level. This seems to confirm the hypothesis that the metastatic genetic profile arises in the primary tumour and 'is maintained in the distant metastases' [32].

## Molecular marker expression in the synchronous group compared to the metachronous group

We hypothesised that the expression of molecular markers in the primaries of the two groups would be different in view of the known clinico-pathological differences that exist between synchronous and metachronous colorectal liver metastases. However, the majority of studies demonstrated no differences in expression. In view, of the limited number of studies comparing the primaries in the two groups as well as the limited number of molecular markers assessed, it is not possible at this stage to draw any firm conclusions.

Cyclooxygenase-2 (COX-2) has been demonstrated to be up-regulated in colorectal adenocarcinomas and correlates with CRC progression and the presence of CLM [33, 34] Nakamato et al. [35] using immunohistochemistry demonstrated no difference in the expression of COX-2 between the two groups. However, Pantaleo et al. [36] using Reverse transcription polymerase chain reaction (RT-PCR) demonstrated that the expression of the COX-2 gene was elevated in the CLM of the synchronous group . Epidermal growth factor receptor plays an important role in the progression and metastatic potential of advanced colorectal cancers [37, 38]. Interestingly, Pantaleo et al. [36] demonstrated using RT-PCR and enzyme-linked immunosorbent assay that EGFR was significantly overexpressed in metachronous group. However, another study using immunohistochemistry found no difference in the expression of EGFR between the two groups [39]. A direct comparison of the findings between the studies assessing COX-2 and EGFR using IHC and RT-PCR cannot be made due to the different laboratory techniques used. Pantaleo et al. [36] findings are interesting and would imply that the synchronous group represent a different biological entity to the metachronous group . However, they cannot be interpreted as significant without further validation. The expression of TGF-α was found to be higher in the liver metastases of the synchronous group compared to the liver metastases of the metachronous group, although this difference was not significant it may denote a biological difference [39].

An important aspect of CLM development is the ability of metastatic tumour cells to evade immunological responses during migration and invasion of the liver. Mature dendritic cells are known to increase in number in response to CLM and increased numbers are associated with a reduced rate of growth of the metastases [40]. One study demonstrated that the metastases in the metachronous group were found to have a significantly higher number of mature dendritic cells [41]. This would suggest that both groups of metastases elicit a different immunological response and could explain the difference in tumour aggression between the two groups [41].

A recent study, demonstrated that a primary tumour in situ in the synchronous group resulted in a higher Ang-1/Ang-2 ratio in the liver metastases compared to either a synchronous group with their primary tumour resected or the metachronous group [42]. It was also demonstrated that the adjacent liver parenchyma in the synchronous group with their primary in situ had significantly higher expression levels of angiogenic factors including VEGF [42]. These findings suggest that the primary tumour has an important role in the progression of colorectal liver metastases by creating a 'permissive soil' for the metastases to proliferate.

These results could lead one to hypothesise that any differences demonstrated between the metastases of the synchronous and the metachronous groups could be related to the presence or absence of the primary tumour.

## Conclusion

The review of the literature confirms that both synchronous and metachronous colorectal liver metastases 'evolve' and exhibit different biological characteristics to their respective colorectal cancer primaries. Although there are conflicting results, the systematic review suggests that biological differences between the liver metastases of the synchronous and metachronous groups may exist and are consistent with the clinically more aggressive nature of synchronous colorectal liver metastases. Whether these differences are as a result of the host immunological response or denote that synchronous or metachronous colorectal liver metastases represent different tumour subtypes remains underdetermined. Determining whether these two groups of patients have biologically distinct metastases is crucial as it could improve and 'tailor' current oncological management according to the timing of the liver metastases presentation. One of the most interesting questions arising from this review is whether any differences at a metastatic level are present at a primary tumour level. Indeed, if differences are detectable within the primary tumour this could have important clinical implications at a pre-operative biopsy stage as well as in routine post-operative surveillance. It is clear that important changes occur within the primary tumour and are maintained throughout the metastatic cascade. In addition, recent evidence seems to suggest that the presence of the primary tumour may have an influence on the biological characteristics of the liver metastases. The review has served to highlight that the main focus of recent research has been to determine whether biological differences exist at a metastatic level and it is has thus not been possible to determine whether differences between the two groups occur at primary tumour stage. Future research should include comparison of the primaries between the two groups.

**Acknowledgments** This review was based on work performed for a MD(Res) degree at Imperial College, London, Division of Surgery and Cancer.

**Conflict of interest** The authors have no conflicts of interest to declare.

## References

1. International Agency for Research on Cancer (IARC), 2012. http://globocan.iarc.fr/factsheet.asp. Accessed 23 Jan 2012
2. Bruin SC, He Y et al (2011) Molecular alterations associated with liver metastases development in colorectal cancer patients. Br J Cancer 105(2):281–287
3. Thomas P, Forse RA, Bajenova O (2011) Carcinoembryonic antigen (CEA) and its receptor hnRNP M are mediators of metastasis and the inflammatory response in the liver. Clin Exp Metastasis 28(8):923–932
4. Bird NC, Mangnall D, Majeed AW (2006) Biology of colorectal liver metastases: a review. J Surg Oncol 94(1):68–80
5. Tan EK, Ooi LL(2010) Colorectal cancer liver metastases–understanding the differences in the management of synchronous and metachronous disease. Ann Acad Med Singapore 39(9):719-33
6. Haddad AJ, Hani MB, Pawlik TM, Cunningham SC (2011) Colorectal liver metastases. Int. J. Surg. Oncol Article ID 285840. doi:10.1155/2011/285840
7. Ghadjar P, Rubie C, Aebersold DM, Keilholz U (2009) The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. Int J Cancer 125(4):741–745
8. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A (eds) (2010) AJCC cancer staging manual (7th ed). Springer, New York
9. Mekenkamp LJ, Koopman M, Teerenstra S, van Krieken JH, Mol L, Nagtegaal ID, Punt CJ (2010) Clinicopathological features and outcome in advanced colorectal cancer patients with synchronous vs metachronous metastases. Br J Cancer 103(2):159–164
10. Dexiang Z, Li R, Ye W, Haifu W, Yunshi Z, Qinghai Y, Shenyong Z, Bo X, Li L, Xiangou P, Haohao L, Lechi Y, Tianshu L, Jia F, Xinyu Q, Jianmin X (2012) Outcome of patients with colorectal liver metastasis: analysis of 1,613 consecutive cases. Ann Surg Oncol 19(9):2860–2868
11. Mantke R, Schmidt U, Wolff S, Kube R, Lippert H (2012) Incidence of synchronous liver metastases in patients with colorectal cancer in relationship to clinico-pathologic characteristics. Results of a German prospective multicentre observational study. Eur J Surg Oncol 38(3):259–265
12. Moher D, Liberati A, Tetzlaff J, Altman DG (2010) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Int J Surg. doi:10.1016/j.ijsu.2010.02.007
13. Howick J, Chalmers I, Glasziou P, Greenhalgh T, Heneghan C, Liberati A, Moschetti I, Phillips B, Thornton H, Goddard O, Hodgkinson M. "The Oxford 2011 Levels of Evidence". Oxford Centre for Evidence-Based Medicine. http://www.cebm.net/index.aspx?o=5653. Accessed 23 Jan 2012
14. Swan PJ, Welsh FK, Chandrakumaran K, Rees M (2011) Long-term survival following delayed presentation and resection of colorectal liver metastases. Br J Surg 98(9):1309–1317. doi: 10.1002/bjs.7527.
15. Wang SC, Lin JK, Wang HS, Yang SH, Li AF, Chang SC (2010) Nuclear expression of CXCR4 is associated with advanced colorectal cancer. Int J Colorectal Dis 25(10):1185–1191
16. Rudmik LR, Magliocco AM (2005) Molecular mechanisms of hepatic metastasis in colorectal cancer. J Surg Oncol 92(4): 347–359
17. Li JQ, Miki H, Wu F, Saoo K, Nishioka M, Ohmori M, Imaida K (2002) Cyclin A correlates with carcinogenesis and metastasis, and p27(kip1) correlates with lymphatic invasion, in colorectal neoplasms. Hum Pathol 33(10):1006–1015
18. Thomas GV, Szigeti K, Murphy M, Draetta G, Pagano M, Loda M (1998) Down-regulation of p27 is associated with development of colorectal adenocarcinoma metastases. Am J Pathol 153(3): 681–687
19. Lloyd RV, Erickson LA, Jin L, Kulig E, Qian X, Cheville JC, Scheithauer BW (1999) p27kip1: a multifunctional cyclin-dependent kinase inhibitor with prognostic significance in human cancers. Am J Pathol 154(2):313–323

20. Li JQ, Miki H, Ohmori M, Wu F, Funamoto Y (2001) Expression of cyclin E and cyclin-dependent kinase 2 correlates with metastasis and prognosis in colorectal carcinoma. Hum Pathol 32(9):945–953
21. Kobayashi H, Sugihara K, Uetake H, Higuchi T, Yasuno M, Enomoto M, Iida S, Azuma M, Mori R, Omori A, Lenz HJ, Danenberg KD, Danenberg PV (2008) Messenger RNA expression of TS and ERCC1 in colorectal cancer and matched liver metastasis. Int J Oncol 33(6):1257–1262
22. Inokuchi M, Uetake H, Shirota Y, Yamada H, Tajima M, Sugihara K (2004) Gene expression of 5-fluorouracil metabolic enzymes in primary colorectal cancer and corresponding liver metastasis. Cancer Chemother Pharmacol 53(5):391–396
23. Ghadjar P, Coupland SE, Na IK, Noutsias M, Letsch A, Stroux A, Bauer S, Buhr HJ, Thiel E, Scheibenbogen C, Keilholz U (2006) Chemokine receptor CCR6 expression level and liver metastases in colorectal cancer. J Clin Oncol 24(12):1910–1916
24. Kim J, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, Foshag L, Bilchik AJ, Hoon DS (2005) Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. J Clin Oncol 23(12): 2744–2753
25. Ghadjar P, Rubie C, Aebersold DM, Keilholz U (2009) The chemokine CCL20 and its receptor CCR6 in human malignancy with focus on colorectal cancer. Int J Cancer 125(4):741–745
26. Wu Y, Jin M, Xu H, Shimin Z, He S, Wang L, Zhang Y (2010) Clinicopathologic significance of HIF-1α, CXCR4, and VEGF expression in colon cancer. Clin Dev Immunol Epub 2010 Oct 7
27. Kim YW, Ko YT, Kim NK, Chung HC, Min BS, Lee KY, Park JP, Kim H (2010) A comparative study of protein expression in primary colorectal cancer and synchronous hepatic metastases: the significance of matrix metalloproteinase-1 expression as a predictor of liver metastasis. Scand J Gastroenterol 45(2): 217–225
28. Esposito DL, Aru F, Lattanzio R, Morgano A, Abbondanza M, Malekzadeh R, Bishehsari F, Valanzano R, Russo A, Piantelli M, Moschetta A, Lotti LV, Mariani-Costantini R (2012) The insulin receptor substrate 1 (IRS1) in intestinal epithelial differentiation and in colorectal cancer. PLoS One 7(4):e36190
29. Bommer GT, Feng Y, Iura A, Giordano TJ, Kuick R, Kadikoy H, Sikorski D, Wu R, Cho KR (2010) Fearon ER.IRS1 regulation by Wnt/beta-catenin signaling and varied contribution of IRS1 to the neoplastic phenotype. J Biol Chem 285(3):1928–1938
30. Ayaki M, Komatsu K, Mukai M, Murata K, Kameyama M, Ishiguro S, Miyoshi J, Tatsuta M, Nakamura H (2001) Reduced expression of focal adhesion kinase in liver metastases compared with matched primary human colorectal adenocarcinomas. Clin Cancer Res 7(10):3106–3112
31. Kuramochi H, Nakajima G, Kaneko Y, Nakamura A, Inoue Y, Yamamoto M, Hayashi K (2012) Amphiregulin and Epiregulin mRNA expression in primary colorectal cancer and corresponding liver metastases. BMC Cancer 13(12):88
32. Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi ZA (2006) Signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. Nat Genet 38(9):1043–1048
33. Yamauchi T, Watanabe M, Kubota T, Hasegawa H, Ishii Y, Endo T, Kabeshima Y, Yorozuya K, Yamamoto K, Mukai M, Kitajima M (2002) Cyclooxygenase-2 expression as a new marker for patients with colorectal cancer. Dis Colon Rectum 45(1):98–103
34. Watanabe T, Kobunai T, Yamamoto Y, Kanazawa T, Konishi T, Tanaka T, Matsuda K, Ishihara S, Nozawa K, Eshima K, Muto T, Nagawa H (2010) Prediction of liver metastasis after colorectal cancer using reverse transcription-polymerase chain reaction analysis of 10 genes. Eur J Cancer 46(11):2119–2126
35. Nakamoto RH, Uetake H, Iida S, Kolev YV, Soumaoro LT, Takagi Y, Yasuno M, Sugihara K (2007) Correlations between cyclooxygenase-2 expression and angiogenic factors in primary tumors and liver metastases in colorectal cancer. Jpn J Clin Oncol 37(9):679–685
36. Pantaleo MA, Astolfi A, Nannini M, Paterini P, Piazzi G, Ercolani G, Brandi G, Martinelli G, Pession A, Pinna AD, Biasco G (2008) Gene expression profiling of liver metastases from colorectal cancer as potential basis for treatment choice. Br J Cancer 99(10):1729–1734
37. Prabhudesai SG, Rekhraj S, Roberts G, Darzi AW, Ziprin P (2007) Apoptosis and chemo-resistance in colorectal cancer. J Surg Oncol 96(1):77–88 Review
38. Yarom N, Jonker DJ (2011) The role of the epidermal growth factor receptor in the mechanism and treatment of colorectal cancer. Discov Med 11(57):95–105
39. De Jong KP, Stellema R, Karrenbeld A, Koudstaal J, Gouw AS, Sluiter WJ, Peeters PM, Slooff MJ, De Vries EG (1998) Clinical relevance of transforming growth factor alpha, epidermal growth factor receptor, p53, and Ki67 in colorectal liver metastases and corresponding primary tumors. Hepatology 28(4):971–979
40. Kito A, Tanaka K, Fujimaki H, Nakazawa M, Togo S, Minami M, Shimada H (2007) Tumor doubling time and local immune response hepatic metastases from colorectal cancer. J Surg Oncol 96(6):525–533
41. Miyagawa S, Soeda J, Takagi S, Miwa S, Ichikawa E, Noike T (2004) Prognostic significance of mature dendritic cells and factors associated with their accumulation in metastatic liver tumors from colorectal cancer. Hum Pathol 35(11):1392–1396
42. van der Wal GE, Gouw AS, Kamps JA, Moorlag HE, Bulthuis ML, Molema G, de Jong KP (2012) Angiogenesis in synchronous and metachronous colorectal liver metastases: the liver as a permissive soil. Ann Surg 255(1):86–94
43. Shirota Y, Ichikawa W, Uetake H, Yamada H, Nihei Z, Sugihara K (2002) Intratumoral dihydropyrimidine dehydrogenase messenger RNA level reflects tumor progression in human colorectal cancer. Ann Surg Oncol 9(6):599–603
44. Shi H, Hood KA, Hayes MT, Stubbs RS (2011) Proteomic analysis of advanced colorectal cancer by laser capture microdissection and two-dimensional difference gel electrophoresis. J Proteomics 75(2):339–351
45. D'Arrigo A, Belluco C, Ambrosi A, Digito M, Esposito G, Bertola A, Fabris M, Nofrate V, Mammano E, Leon A, Nitti D, Lise M (2005) Metastatic transcriptional pattern revealed by gene expression profiling in primary colorectal carcinoma. Int J Cancer 115(2):256–262
46. Goldstein NS, Armin M (2001) Epidermal growth factor receptor immunohistochemical reactivity in patients with American Joint Committee on Cancer Stage IV colon adenocarcinoma: implications for a standardized scoring system. Cancer 92(5):1331–1346
47. Al-Mulla F, Keith WN, Pickford IR, Going JJ, Birnie GD (1999) Comparative genomic hybridization analysis of primary colorectal carcinomas and their synchronous metastases. Genes Chromosomes Cancer 24(4):306–314
48. Takahashi Y, Ishii Y, Nishida Y, Ikarashi M, Nagata T, Nakamura T, Yamamori S, Asai S (2006) Detection of aberrations of ubiquitin-conjugating enzyme E2C gene (UBE2C) in advanced colon cancer with liver metastases by DNA microarray and two-color FISH. Cancer Genet Cytogenet 168(1):30–35
49. Kuramochi H, Hayashi K, Uchida K, Miyakura S, Shimizu D, Vallböhmer D, Park S, Danenberg KD, Takasaki K, Danenberg PV (2006) Vascular endothelial growth factor messenger RNA expression level is preserved in liver metastases compared with corresponding primary colorectal cancer. Clin Cancer Res 12(1):29–33

50. Ono M, Sakamoto M, Ino Y, Moriya Y, Sugihara K, Muto T, Hirohashi S (1996) Cancer cell morphology at the invasive front and expression of cell adhesion-related carbohydrate in the primary lesion of patients with colorectal carcinoma with liver metastasis. Cancer 78(6):1179–1186
51. Nanashima A, Yamaguchi H, Yasutake T, Sawai T, Kusano H, Tagawa Y, Nakagoe T, Ayabe H (1997) Gain of chromosome 20 is a frequent aberration in liver metastasis of colorectal cancers. Dig Dis Sci 42(7):1388–1393
52. Kitabatake T, Kojima K, Fukasawa M, Beppu T, Futagawa S (2002) Correlation of thymidine phosphorylase staining and the Ki-67 labeling index to clinicopathologic factors and hepatic metastasis in patients with colorectal cancer. Surg Today 32(4):322–328
53. Konishi K, Watanabe Y, Shen L, Guo Y, Castoro RJ, Kondo K, Chung W, Ahmed S, Jelinek J, Boumber YA, Estecio MR, Maegawa S, Kondo Y, Itoh F, Imawari M, Hamilton SR, Issa JP (2011) DNA methylation profiles of primary colorectal carcinoma and matched liver metastasis. PLoS One 6(11):e27889
54. Petrowsky H, Sturm I, Graubitz O, Kooby DA, Staib-Sebler E, Gog C, Köhne CH, Hillebrand T, Daniel PT, Fong Y, Lorenz M (2001) Relevance of Ki-67 antigen expression and K-ras mutation in colorectal liver metastases. Eur J Surg Oncol 27(1):80–87
55. Chen J, Li Q, Wang C, Wu J, Zhao G (2010) Prognostic significance of c-erbB-2 and vascular endothelial growth factor in colorectal liver metastases. Ann Surg Oncol 17(6):1555–1563
56. Kochhar R, Halling KC, McDonnell S, Schaid DJ, French AJ, O'Connell MJ, Nagorney DM, Thibodeau SN (1997) Allelic imbalance and microsatellite instability in resected Duke's D colorectal cancer. Diagn Mol Pathol 6(2):78–84
57. Nanashima A, Shibata K, Nakayama T, Tobinaga S, Araki M, Kunizaki M, Takeshita H, Hidaka S, Sawai T, Nagayasu T, Yasutake T (2009) Clinical significance of microvessel count in patients with metastatic liver cancer originating from colorectal carcinoma. Ann Surg Oncol 16(8):2130–2137
58. Kahlert C, Lahes S, Radhakrishnan P, Dutta S, Mogler C, Herpel E, Brand K, Steinert G, Schneider M, Mollenhauer M, Reissfelder C, Klupp F, Fritzmann J, Wunder C, Benner A, Kloor M, Huth C, Contin P, Ulrich A, Koch M, Weitz J (2011) Overexpression of ZEB2 at the invasion front of colorectal cancer is an independent prognostic marker and regulates tumor invasion in vitro. Clin Cancer Res 17(24):7654–7663