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Prognostic and predictive markers in liver limited stage IV colorectal cancer

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ABSTRACT

Colorectal cancer is the third most commonly diagnosed cancer among both men and women. Personalised treatment options remain complex, although there is broad agreement over which patients with colorectal liver metastases (CRLM) should and should not be offered resection. Decisions on an optimal management strategy involves careful assessment of both technical and oncological factors. In this review we aim to summarise current prognostic biomarkers for metastatic colorectal cancers, specifically patients considered for resection.

A number of clinico-pathological factors have been identified as prognostically important with good internal validity, but limited external validity. Furthermore, these prognostic scoring systems do not take factor in modern chemotherapeutic agents and the disease modification these agents produce. Histopathological response to chemotherapy is of significant prognostic importance.

Molecular markers can help predict the efficacy of a biological agent. An important prognostic factor of liver metastasis is the recognition that location of the primary colorectal cancer impacts on metastatic phenotype and represents difference in genotype, i.e. proximal tumours are more aggressive than distal tumours with an increased likelihood of disease progression.

Several mutational molecular markers identified include microsatellite instability, BRAF, and KRAS/NRAS and combination mutations, which confer poorer outcomes.

Accurate prognostication in patients with liver limited colorectal metastases remains crucial, as this allows tailoring treatment options to each disease and improving outcomes. Access to tissue before treatment remains a limitation although advances in ability to assess tumour biology by non-invasive methods are promising.

1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer among both men and women, and second most common cause of death in the UK. In 2018, the global incidence was approximately 1.8 million cases, and accounted for almost 700,000 deaths, making it the fourth leading cause of cancer death worldwide [1]. In the United Kingdom, there were more than 41,000 new cases and nearly 16,000 deaths. Since 2001, there has been a steady decrease in mortality, attributed to advances in early detection and improved therapy. Metastatic spread occurs in approximately 60% of patients, with a median overall survival (OS) for metastatic colorectal cancer (mCRC) of approximately 24–27 months, and a 5-year survival of 10–15% [2]. For a subset of patients with surgically resectable metastatic disease, resection with curative intent provides the best opportunity for cure with an improved median OS of 40–55 months. Combined with multimodal systemic therapy, 5 year survival for these patients can reach 40% [2].

Selecting the optimal treatment for each individual treatment remains complex. There is broad agreement over which patients with colorectal liver metastases (CRLM) clearly should and should not be offered resection [3,4]. For a patient with a small solitary liver metastasis presenting several years after primary resection, surgery is generally accepted to offer a clear and significant long-term survival benefit. For patients with synchronous extensive large volume disseminated disease, surgery is unlikely to improve outcome. Deciding on the optimal management strategy for patients who fall into the grey area between these extremes remains one of the most challenging decisions in the management of stage

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IV colorectal cancer, and involves a careful assessment of both technical and oncological factors.

This review aims to summarise current prognostic biomarkers for mCRC, specifically patients considered for resection.

2. Existing clinico-pathological prognostic biomarkers

Since the late 1990s, a number of clinico-pathological factors have been identified as prognostically important. Fong et al. identified the need for widely applicable clinical criteria to select patients who may benefit from hepatic resection for mCRC and published their scoring system. The criteria included nodal status of the primary tumour (positive vs negative), disease-free interval from primary tumour to liver metastasis, number of tumours, level of carcino-embryonic antigen (CEA) at time of hepatectomy, and size of the largest tumour [5].

Other scoring systems have been developed over the years and have included various clinicopathological factors. These include: nodal status of primary tumour (positive nodes and number of positive nodes), disease-free interval from primary tumour, number of tumours, size of largest hepatic tumour, CEA level at time of hepatectomy, age, T stage and N stage of primary tumour, clearance of primary tumour, resectable extra-hepatic distant metastasis, differentiation grade, need for perioperative blood transfusion, neutrophil to lymphocyte ratio, t-cell tumour infiltration and sex [6]. Despite the vast number of prognostic scoring indexes available, these were based on historical series, before the era of modern perioperative chemotherapy. The limitations of such risk scoring systems include their lack of reproducibility when applied in other institutions [7]. The advent of new biological agents and their use in advanced colorectal disease do not account for the variable modification to the disease these agents produce. Histopathological response to neoadjuvant treatment [8] and adjuvant chemotherapy for metachronous primary disease [9] are of significant prognostic importance.

There is now a growing interest in direct assessment and integration of tumour biomarkers into stratification systems to better refine prognostication [6].

3. Biomarkers for liver limited stage IV colorectal cancer

Knowledge of the molecular development of CRC has improved. Molecular markers can help predict the efficacy of a biological agent. They can be prognostic, predictive or both. Prognostic markers do not determine the specific choice of a therapy, only likely long-term outcome, whilst predictive markers predict differential efficacy helping guide the choice of therapy. An important factor in the prognostication of liver metastasis is the growing recognition that location of the primary colorectal cancer impacts on metastatic phenotype and represents difference in genotype. Kirsten rat sarcoma 2 viral oncogene (KRAS) mutations have been found to be twice as common in lesions proximal to the splenic flexure [10]. This finding was reinforced when it was noted frequencies of CpG island methylator phenotype-high (CIMP), microsatellite instable-high (MSI), and BRAF mutation gradually decreased from ascending colon to rectum. Proximal disease is therefore seemingly more aggressive, with proximal tumours who develop metastatic recurrence less likely to be treatable with liver resection than distal disease [6,11–13].

4. Prognostic molecular markers

4.1. RAS

The most robust and clinically useful biological marker in patients with mCRC is RAS mutational status. KRAS and NRAS are proteins and downstream effectors of EGFR, with binding of the EGF ligand to the receptor triggering downstream signalling via the PI3K/AKT/MTOR and RAF/MEK/ERK cellular proliferation pathways. Upstream signals activate wild-type KRAS by promoting the exchange of bound GDP for GTP. However, mutated RAS corrupts this process by preventing GAPs from promoting GTP hydrolysis by RAS, subsequently causing RAS proteins to accumulate in the GTP-bound active form.

Mutant RAS is found in about 35–45% of CRCs [14–16], with 95% of all mutation types occurring on codon 12 (80%) and codon 13 (15%). Less commonly occurring mutations occur on codons 61, 146 and 154 [17]. They are almost all single nucleotide point mutations, and the most common patterns are G12D, G12A, G12R, G12C, G12S, G12V and G13D. In the codon 12 mutation, pG12D and pG12V are the most frequent, and in codon 13, substitution of glycine for aspartate (p.G13D) is the most frequent [18].

Mutant KRAS is known to confer poorer prognosis. Not limited to just liver, Yaegar et al. reported similar results within 918 patients with mCRC [19]. They showed improved survival in wild type KRAS patients when compared to mutant KRAS patients (81 months vs 47 months respectively); $p < 0.001$) and concluded the varying metastatic potential CRC has depending on its KRAS mutation status (higher incidence of lung, brain and bone metastasis). Karagkounis et al. reported significantly worse median overall survival (45.2 vs 71.9 months) and 5-year overall survival (49.8% vs 57.4%, $p = 0.007$) when carrying mutated KRAS as opposed to wild type KRAS in resected CRLM [14].

A recently published systematic review and meta-analysis evaluating prognostication of mutated KRAS concluded there is a negative association with OS and relapse-free survival in patients who undergo complete liver resection for colorectal liver metastasis (HR 1.674, [1.341–2.089]; $p < 0.001$ and HR 1.529, [1.287–1.817]; $p < 0.001$ respectively) [20].

Recently, Margonis et al. expanded upon optimal resection margins in mutated KRAS patients. Classically, surgeons aim for an R0 resection, i.e. a 1 cm circumferential rim around the macroscopically visible metastatic lesion [11,21]. This is despite current literature being inconclusive regarding optimal margin width, with some authors suggesting an R1 resection had no impact on overall survival [22,23] whilst others concluded that even a submillimetre margin improved overall survival compared to an R1 resection [24]. Margonis et al. compared tumour biology and the effect of resection margin on overall survival with a specific focus on the importance of KRAS status. They identified that in mutant KRAS a wider resection margin did not confer improved overall survival. Similarly, with wild type KRAS tumours, a margin clearance of 1–4 mm or more did not offer improved OS compared to >4 mm [25].

To date, only two scoring system take into account RAS status for prediction of survival after resection of CLM [26]. Zimmitti et al. analysed the predictive impact of RAS mutations using either radiologic morphological response (model 1) or pathologic response (model 2) in patients undergoing chemotherapy before CRLM resection. Using multivariate analysis, they confirmed that wild-type RAS was a strong predictor of morphological (OR 4.38, [1.45–13.15]; $p = 0.008$) and pathological (OR 2.79, [1.29–6.04]; $p = 0.009$) response. They noted RAS mutations independently correlated with both OS and DFS (HR 3.57 and 2.30, respectively, in model 1, and 3.19 and 2.09, respectively, in model 2). They showed mutational RAS status, as a predictor of OS and PFS, can be used to complement the current prognostic indicators for patients undergoing curative resection of CRLM after pre-operative modern chemotherapy [26]. Brudvik et al. created a modified version of the most widely used score – the Memorial Sloan Kettering Cancer Centre clinical score. A multivariate analysis adding RAS mutation

status to the traditional score revealed the only factors that were statistically significant to predict OS were node-positive primary tumour ($p < 0.001$), largest liver metastasis (>50 mm in diameter) ($p = 0.031$) and RAS mutation ($p < 0.001$). The score was validated in an international multicentre cohort and outperformed the traditional score at stratifying patients by OS [27].

4.2. Microsatellite instability

Impaired DNA mismatch repair (MMR) results in genetic hypermutability, known as microsatellite instability. It consists of insertion and deletion mutations in short tandem repeats as well as nucleotide substitutions throughout the genome. The consensus molecular subtypes (CMS) of CRC have been defined recently into 4 categories: CMS1 (microsatellite instability immune subtype: hypermutated subtype of CRC, microsatellite unstable with a strong immune activation); CMS2 (canonical subtype of CRC: epithelial subtype with upregulation of the WNT and MYC signalling pathways); CMS3 (metabolic subtype of CRC: epithelial subtype with metabolic dysregulation); and CMS4 (mesenchymal subtype of CRC with prominent transforming growth factor-b activation, stromal invasion and neoangiogenesis) [28]. Based on the presence of mononucleotide (BAT25 and BAT26) and dinucleotide (D2S123, D5S346 and D17S250) repeating units, genomic instability can be classified into two different subtypes: MSI-high (MSI-H) and MSI-low (MSI-L) [29].

MSI-H CRC accounts for 15% of all CRC and includes hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome and sporadic (12%). MLH1, MSH2, MSH6, PMS2 or TACSTD1 represent genes within the MMR system which can mutate causing the inability to repair DNA mismatches [30]. MSI-H tumours are more commonly located in the right colon and are histologically typified by poor differentiation, mucinous features and lymphocytic invasion. Compared to microsatellite stable tumours, MSI-H tumours favour decreased risk of distant recurrence [31,32].

4.3. BRAF

BRAF plays an important role in the epidermal growth factor receptor (EGFR)-mediated mitogen-activated protein kinase (MAPK) pathway. Within this, serine/threonine protein kinase is activated by the RAS small GTPase [33]. Mutated BRAF is found activated in 15% of all known cancer types. Mutated BRAF tumours have similar characteristics of MSI-H tumours: more often right sided, higher grade, associated with MSI and in older age [34,35]. Contrary to the predictive role of mutated BRAF, its prognostic role is well defined, and attributed with a significantly poorer prognosis. In a study of more than 1200 patients, BRAF mutation was associated with significantly worse overall survival (hazard ratio (HR) 1.78 [1.15–2.76]; p-value 0.01) [36]. Tran et al. reported patients with mutated BRAF CRCs had a significantly worse median OS (10.4 months) when compared with patients who wild-type BRAF CRCs (34.7 months, $p < 0.01$) [37].

BRAF mutations are identified in approximately 40–60% of sporadic MSI-H tumours, but BRAF-inhibitors are not particularly effective for patients with stage IV CRC with the MSI-H genotype, with a response rate of approximately 5% [38]. A proposed mechanism of mutation has been described as alterations of proteins in the downstream activation of mitogen activating protein kinase pathway, such as KRAS (and downstream PI3K), RAF, MEK and ERK. Another includes feedback regulation of EGFR (CD25c) [38,39]. Whilst BRAF mutations in general are associated with more aggressive tumour biology, the role of specific codon mutations has only recently been characterised. Described in up to 80% of all BRAF mutations, the V600E (1799T > A) substitution is by far the most common [40]. Margonis et al. reported the association between V600E BRAF mutations and worse OS (HR 2.76, [1.74–4.37]; $p < 0.001$) and DFS (HR 2.04, [1.30–3.20]; $p = 0.002$) [41].

4.4. Combination mutations

Whilst certain single mutations confer poorer outcomes than others, it is also important to consider a combination of mutations in a single tumour, as well as mutations in multiple tumours. Although the potential prognostic and predictive importance of such mutations are recognised, existing evidence is limited. Chun et al. sequenced 50 cancer-related genes from tumours in 401 patients undergoing CLM resection and identified the double mutation of TP53/RAS to be associated with primary right colonic tumours. This double mutation independently prognosticated decreased survival after resection (HR 2.62, [1.41–4.87]; $p = 0.002$) [42]. Loes et al. also studied the effect of combination mutation panels and heterogeneity. When comparing intertumoral heterogeneity affecting KRAS, BRAF, PI3K or TP53, a homogenous mutation and patients with no mutations, they found a significantly reduced disease specific survival in intertumoral heterogeneity (median 18 vs. 37 vs. 58 months, $p < 0.001$) [43].

4.5. Histopathological growth pattern

Histopathological growth patterns define the encroachment of the tumour onto surrounding normal tissue. The role of histopathological patterns (HGPs) for prognostication has been validated. Three primary HGPs were identified and termed desmoplastic (d), replacement (r) and pushing (p) pattern [44]. A number of studies have all correlated HGP to prognosis [45–47]. Eefsen et al. found patients non dHGP had inferior recurrence free survival when compared to patients displaying desmoplastic HGP (HR 2.16, [1.29–3.62]; $p = 0.003$), and when displaying replacement HGP, a significant worse OS was seen (HR 2.26, [1.16–4.38]; $p = 0.016$) [48].

The most recent study by Galjart et al. showed that in patients undergoing their first resection of CRLM and who are chemo-naïve, the presence of pure dHGP signifies improved survival (OS HR 0.661, [0.484–0.902]; $p = 0.009$; PFS HR 0.671, [0.519–0.867]; $p = 0.002$), and the presence of any non dHGP indicates impaired prognosis [49].

4.6. Inflammatory biomarkers

There has been a recent increase in interest over the use of inflammatory response of the host to the tumour as a prognostic indicator. Mantovani et al. described the pathways linking inflammation and tumourigenesis [50]. Succinctly, the modulation of inflammatory cells and mediators allows for neoangiogenesis (neutrophils and platelets), and coupled with remodelling of extracellular matrix promotes metastatic spread. Lymphocytes have an anti-tumour effect and mediate the host response, and monocytes, with tumour-associated macrophages, are part of the myeloid cell lineage promoting tumour growth [51–53]. On this basis, several studies have analysed the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR). In a retrospective study, Riedl et al. found that elevated levels of Interleukin 6, CRP and the NLR were prognosticators of impaired disease outcome, but high levels of LMR were associated with prolonged survival [54]. Whilst single cut-off values of NLR, PLR and LMR have been identified as 2.2–5, 150–300 and 2.14–3.78 respectively [55–57], the values were not externally validated by Dupré et al. [58]. Despite a further recommendation of investigative work in larger cohorts, the lack of

inflection point or consistency amongst the ratios suggest the associations are likely due to chance.

5. Predictive molecular markers

5.1. KRAS/NRAS

The role of KRAS as a predictive biomarker for anti-EGFR therapy is well recognised. This was first validated by Lièvre et al. who showed KRAS-mutated patients derived no benefit from cetuximab and had a poorer OS independent of treatment compared to wild-type KRAS patients [59]. Multiple studies have further reinforced this finding. Vauthey et al. evaluated patients who had received neoadjuvant chemotherapy (single regimen of doublet oxaliplatin or an irinotecan-based chemotherapy) prior to hepatectomy with curative intent. In 193 resected patients, they found KRAS mutations in 22.3% (markedly lower than the reported rate in all mCRC patients of around 35–45%) and showed these were associated with worse progression-free survival (PFS) (HR 1.9, [1.2–3.0]; $p = 0.005$) and OS (HR 2.3, [1.1–4.5]; $p = 0.002$) after surgery [16]. At a median follow up of 33 months, three-year OS was 81% in wild type RAS compared with 52.2% in mutant RAS. Five-year OS was 65.4% compared to 44.7% respectively. Interestingly, both Yaeger et al. and Vauthey et al. observed a higher incidence of lung recurrence with mutated KRAS (HR 1.52, [1.1–1.92]; $p < 0.01$ and 2.0, [1.1–3.4]; $p = 0.01$ respectively).

5.2. Anti-EGFR therapy biomarkers

Whilst RAS status, BRAF activating mutations and MSI have all proven to be excellent biomarkers, searches remain to find further biomarkers which will help in the predicting response to anti-EGFR therapy.

Two further mutations in the PI3KCA and TP53 pathway have had variable success. Mutated PI3KCA (the gene encoding PI3K, controlling cell proliferation and survival), resulting in aberrant AKT activation, was originally thought to correlate with lack of response to anti-EGFR therapy, but with inconsistent study results, its reliability is limited [60,61]. The TP53 mutation, which switches off cell apoptosis, and may indicate success of anti-EGFR therapy, is more promising, although data for this is limited and requires further studies [62].

In a biomarker study, from the PICCOLO trial, Seligmann et al. looked at the role of ligand expression as a predictive marker for immunotherapy. Levels of epiregulin and amphiregulin (RNA expression of EGFR ligands) were measured and they found that high ligand expression levels were predictive for benefit from immunotherapy in RAS wild type patients (HR 0.38, [0.24–0.61]; $p < 0.001$) [63].

5.3. Microsatellite instability

MSI, also a prognostic biomarker, can also be used as a predictive biomarker in checkpoint immunotherapy. Le et al. showed that PD-1 (programmed death) pathway inhibitors were more efficacious in mismatch repair-deficient tumours compared to mismatch repair-proficient tumours [64]. This was on the basis of high expression levels of checkpoint proteins in cancers with MSI-H. MSI-H is not specific to colorectal cancer, but is independent of tumour histology and dependent on the tumour's genetic composition [65]. This has led to the development of drugs treating mismatch status, rather than location of tumour [66]. Although no drugs have yet been approved for use in colorectal cancer, trials are ongoing. Testing for MSI-H may have a predictive value for the use of PD-1 and programmed cell death ligand-1 inhibitors, which the ongoing studies and trials will show.

6. Conclusions

The need for accurate prognostication in patients with liver limited colorectal metastases remains crucial. Although surgery offers enormous potential benefits in selected patients, it is clearly not appropriate for all, and so better prognostication and prediction of treatment response are essential to improve outcomes. Current approaches to stratified therapies are limited by access to tissue prior to treatment. Advances in our ability to assess tumour biology by non-invasive methods (radiomic biomarkers and liquid biopsies) offer great promise, as well as the potential to perform longitudinal assessment in response to therapies. Future work should focus on applying these new technologies to better stratify patients for treatment in what is an extremely heterogenous population.

Whilst the need for accurate prognostication is crucial to develop personalised medicine, their role is limited. Tumour biology should not preclude patients with technically resectable disease. Biomarkers should be used to aide adjuvant therapy choice as well as provide prognostication for patients.

Statements

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Author contributions

Adeeb Rehman wrote the main body of the manuscript. Help was provided by Robert Jones for the introduction and areas relating to molecular pathways. This was overseen by Graeme Poston and corrections/amendments made on his recommendation.

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