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Predicting the outcome of chemotherapy for colorectal cancer

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Colorectal cancer is the second leading cause of cancer-related deaths in the Western world. Recently, improvements have been made in treating patients with advanced colorectal cancer; however, response rates still remain low at only 40–50% following combination therapy. The major limitation in treating these patients is the development of drug resistance. Therefore, there is a need to identify which patients will respond to a given chemotherapy regime so that they will be spared the unnecessary time and toxicity of being placed on a regime from which they will derive no benefit. It is also widely accepted that exposure to these chemotherapies themselves can induced acute resistance. Recent developments have been made in predicting response to chemotherapy using global approaches, with the ultimate aim of individualising patient treatment and improving overall survival rates.

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Introduction

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths in the Western world. Approximately 75% of patients present with disease localised to the colon or rectum; in stage II or Dukes' B tumours, there is no associated regional lymph node involvement, whereas in stage III or Dukes' C tumours the regional lymph nodes are involved with tumour. In patients with resectable stage III CRC, adjuvant therapy has been demonstrated to improve disease-free survival and overall survival by 35% and 22%, respectively. Yet the role of adjuvant therapy in stage II CRC still remains controversial. The five-year survival for patients with stage II CRC is 75%, demonstrating that the majority of patients are cured by surgery alone. However, 40% of these patients will develop recurrent disease within their lifetime; hence there is a need to identify which of these patients would benefit from adjuvant therapy.

In the past decade, the median survival for patients with metastatic CRC has nearly doubled from 12 months to 22 months. In the metastatic setting, single agent 5-fluorouracil (5-FU) produces a response rate of only 10–20%. As such, 5-FU has recently been combined with the newer agents oxaliplatin and irinotecan, and this has significantly improved response rates to 40–50% [1,2]. The novel biological agents cetuximab (an epidermal growth factor receptor [EGFR] inhibitor) and bevacizumab (a vascular endothelial growth factor [VEGF] inhibitor) have recently been shown to provide additional clinical benefit for patients with metastatic CRC [3,4]. Despite these improvements, there are still a significant number of patients who do not benefit from treatment; hence, there is a need to identify novel panels of molecular and biochemical markers that can be used to predict which patients will respond to traditional and novel therapies.

Several groups have begun to identify panels of predictive markers that correlate with the response to a given therapy [5,6]. If the aim of predictive marker testing is realized, patients will begin to be treated in an individualised way based on their individual tumour profile instead of receiving a standard chemotherapy regime. In CRC, predictive marker testing will be important for two reasons: to identify early stage CRC patients who would benefit from adjuvant chemotherapy; and to identify subgroups of patients with advanced disease who will either respond or not to particular chemotherapy agents.

Chemotherapeutic drugs and potential predictive markers

5-FU

5-FU belongs to a class of drugs known as the antimetabolites. It exerts its effects through inhibition of the nucleotide synthetic enzyme thymidylate synthase (TS) by its active metabolite fluorodeoxyuridine monophosphate, resulting in thymidylate depletion which, if prolonged, causes apoptosis via the so-called thymineless death [7]. 5-FU causes misincorporation of nucleotides into both DNA and RNA, and the following mechanisms have all been implicated in resistance to 5-FU.

Firstly, the primary mechanism of resistance to fluoropyrimidines is an increase in TS expression [8]. The majority of studies evaluating TS as a marker of response to 5-FU have shown that low tumoral TS expression is associated with improved response to 5-FU [9–11], whereas high TS levels correlate with resistance to

5-FU. Moreover, in the locally advanced disease setting, low TS is associated with improved disease-free and overall survival times [12].

Secondly, thymidine phosphorylase converts 5-FU to fluorodeoxyuridine, which can then be converted to the active metabolite fluorodeoxyuridine monophosphate. It has been shown that tumours with high TP expression are less likely to respond to 5-FU [13,14].

Thirdly, dihydropyrimidine dehydrogenase (DPD) catalyses the rate-limiting step in the catabolism of fluoropyrimidines, thereby limiting the bioavailability of 5-FU [15]. Several studies have demonstrated that patients with low DPD expression have longer disease-free survival and improved overall survival compared with those with high levels of DPD [16]. In addition, tumoral DPD has been reported to be an important determinant of response to 5-FU both *in vitro* [17] and *in vivo* in the metastatic setting [18].

Finally, mutations in *p53* [19] and overexpression of *p53* (as a surrogate marker for *p53* mutation) [20–22] have been correlated with response to 5-FU and resistance, respectively. However, conflicting results [23–25] limit the use of *p53* as a predictive marker of 5-FU response.

Interestingly, a study by Salonga *et al.* [18] examined the combined levels of TS, DPD and TP in a series of colorectal tumours treated with 5-FU. Tumours that responded to 5-FU-based therapy had expression values for all three genes (*TS*, *DPD* and *TP*) that were below the non-responsive cut-off levels, which resulted in this group of patients having an overall response rate of 92%. Those patients whose tumours did not respond had high levels of gene expression for at least one of the markers. This underscores the need to test for multiple markers, as it is unlikely that a single marker can accurately predict response to chemotherapy in every patient.

Oxaliplatin

Oxaliplatin is a third-generation platinum compound with a 1,2-diaminocyclohexane side-chain. Oxaliplatin is thought to form a positively charged species that cross-links DNA and eventually leads to cytotoxicity [26]. Several mechanisms are thought to be implicated in the resistance to platinum compounds.

Enhanced DNA repair

Excision repair cross-complementing 1 (ERCC1) is involved in removing bulky helix-distorting adducts produced by oxaliplatin treatment. It has been shown that low *ERCC1* gene expression levels have correlated with improved overall survival after combined 5-FU + oxaliplatin therapy in patients with advanced CRC refractory to first-line chemotherapy [27]. Furthermore, an independent study has demonstrated that both low *TS* and low *ERCC1* mRNA expression is associated with significantly improved survival in patients treated with 5-FU + oxaliplatin [27].

Decreased drug accumulation

Platinum compounds become conjugated to glutathione, which facilitates their export from the cell by either the glutathione conjugate export pump or the multidrug resistance-associated protein. The reaction is catalysed by glutathione-S-transferase enzymes, and glutathione-S-transferase-P1 in particular has been shown to be over-expressed in CRC tissues [28].

Drug inactivation

As highlighted above, oxaliplatin is inactivated by thiol-containing proteins such as glutathione and glutathione-related enzymes [29].

Enhanced tolerance to platinum-DNA adducts

The mismatch repair (MMR) system binds to DNA with cisplatin adducts, but not oxaliplatin adducts. This is probably a result of the non-polar diaminocyclohexane side-chain preventing the MMR system from recognising the lesion and being able to repair it [29].

Irinotecan

Irinotecan is a DNA topoisomerase I inhibitor that is converted to 7-ethyl-10-hydroxy-camptothecan (SN-38) by carboxylesterases [30]. SN-38 exerts its cytotoxicity by trapping the complexes formed by topoisomerase I with DNA, generating single-strand breaks that eventually result in a double-strand break [31]. Several mechanisms of action have been implicated in the resistance to irinotecan: firstly, UGT1A1 glucuronidates SN-38 to form the more polar and inactive glucuronide, which is eliminated in bile and urine [32]; and secondly, a positive relationship could exist between topo-1 activity and cellular sensitivity to irinotecan[33], but this has not yet been proven.

Cetuximab

Many studies have demonstrated that EGFR is over-expressed in approximately 70% of CRC patients [34]. EGFR plays an integral role in cell survival signaling, and therefore is an important target in anti-cancer treatment. Antibodies such as panitumanab and cetuximab (C225 or erbitux) bind competitively to the extracellular domain of EGFR, inhibiting EGF binding and receptor autophosphorylation [35]. These antibodies might also block the production of pro-angiogenic factors such as VEGF and interleukin-8 [36]. A number of markers have been examined, including EGFR and VEGF, as predictors of response to cetuximab; however, these biomarkers have not shown any association with response.

Bevacizumab

Many solid tumours secrete high levels of VEGF, which promotes their vascularisation and initiates formation of metastases [37]. Increased VEGF expression correlates

with advanced tumour stage and poorer prognosis in CRC [38]. Bevacizumab, which is a recombinant humanized monoclonal antibody against VEGF, is now standard care for first-line treatment of metastatic CRC [4,39,40]. The antibody inhibits the binding of VEGF to its endothelial cell receptors. A study by Ince *et al.* [41] attempted to correlate *k-ras*, *b-raf* and *p53* with response to bevacizumab; however, their results were not significant and to date no markers of response to bevacizumab have been identified.

Lack of implementation

The biological markers that have been discussed above have not been implemented for use in the clinical arena. A major reason for this is the lack of a comprehensive and integrated approach to these studies. In terms of the studies discussed above, many have had no defined protocols, no defined primary end-points, no clear analysis plan and the sample size is often insufficient to power the study. In order to implement reliable biological markers in the clinic, these studies need be carried out in a prospective manner, clearly defining the marker prevalence and the sample size needed based on the marker prevalence and using a sensitive and reproducible bioassay [42]. Only if studies are carried out using this focused and disciplined approach will more current and novel predictive markers successfully progress into routine clinical use.

Multiple marker studies

More recently, several studies have begun to focus on high-throughput methodologies such as proteomic profiling, microarray-based gene expression profiling, comparative genomic hybridisation (CGH) analysis and metabolomic profiling, all of which enable tumour samples to be profiled on a global scale. This has major implications for the diagnostic capability and prognostic classification of tumours, with the potential to allow us to predict the response of each individual tumour to chemotherapy. Whereas microarray expression profiling of CRC has been preformed, no comparable protein analysis has been reported. However, it is important to investigate the proteomic profile, as mRNA levels might not correlate with the amount of active protein within the cell. Furthermore, the gene sequence does not describe the post-translational modifications that could be essential for protein function and activity; finally, the study of the genome does not provide information on dynamic cellular processes [43]. CGH identifies specific chromosomal regions that are consistently gained or lost at a high frequency within CRC and has demonstrated an increase in the genetic grade of a tumour with disease progression [44,45]. In CRC, CGH will be a powerful tool to identify whether a correlation exists between a specific chromosomal aberration and patient survival [46].

The most frequently used genome-wide approach in CRC is DNA microarray profiling. Mariadason *et al.* [47] carried out gene expression profiling on 30 CRC cell lines and correlated this with 5-FU sensitivity using three different assays of response. They were able to identify panels of genes that correlated with 5-FU sensitivity and further used 'leave-one-out' cross validation to demonstrate that these genes were predictive for 5-FU response. They noted that this gene set had a greater power to predict response than did the four 'classical' determinants of 5-FU response: *TS*, *TP*, *p53* and MMR status. From this study, they were able to identify two other gene sets that correlated with sensitivity to either camptothecan or oxaliplatin [47]. The limitations of this study are, firstly, that it involves *in vitro* data and, secondly, it needs to be independently tested in blinded samples. It would be of great interest to discover whether these *in vitro* classifier sets could be translated to the clinical setting to predict response to chemotherapy in patients.

To date, clinical studies have been performed that predict for response to chemotherapy in breast, bladder and ovarian cancer. Such studies have not yet been completed in CRC; however, several studies have recently been conducted that aim to predict diagnosis or prognosis of CRC. An important study in this area is that of Wang *et al.* [48••] who used gene expression profiling to identify markers of recurrence for stage II CRC. Using two supervised class prediction approaches, they identified a 23-gene set and a 60-gene set. Further analysis revealed that only the 23-gene set was predictive for CRC. This gene set was validated in 36 independent patients and demonstrated an overall accuracy of 78% [48••]. This study would benefit from increasing the number of samples in both the training and test sets to increase the predictive power of the model. In addition, this report highlights the need to carefully select the correct analysis for the purpose of the test. Eschrich *et al.* [49••] used gene expression profiling to identify a classifier set that could distinguish a good prognosis from a poor prognosis. A crucial component of this study was that it was validated in an independent test set from a Danish colon cancer dataset, and demonstrated high predictive accuracy. It was suggested that this classifier set could identify patients with a poor prognosis who would benefit from adjuvant treatment and, furthermore, that this outperformed Dukes' staging [49••]. Finally, Barrier *et al.* [50••] aimed to use gene expression profiling to identify stage II and III patients who are at higher risk of recurrence. Interestingly, an important facet of this study was that it used both tumour and non-neoplastic mucosa to derive a predictive marker set, as there was evidence to suggest that interactions occur between the stromal and the cancer cells and that these are prerequisite for metastasis. The authors conclude that it is possible to build a prognostic predictor from either the tumour or the non-neoplastic mucosa; however, the model built from the non-neoplastic mucosa shows a greater degree of stability, possibly owing to the homogeneity of the samples [50••].

This is an important study as it clearly demonstrates that it is possible to build a predictive model from sites other than the primary tumour.

Conclusions

This review has aimed to discuss the previously identified individual markers of response to chemotherapy and the reasons why they have not been employed. Salonga *et al.* [18] previously demonstrated how a small number of genes can exert major effects on drug response, but it is likely that the combined identification of polymorphic genes, proteins, chromosomal aberrations and metabolites will ultimately lead to the ability to predict enhanced response to chemotherapy while minimising drug toxicity. It is also extremely important to test these markers using a disciplined and standardised approach in a prospective manner in order for these markers to be implemented in the clinic [42].

In order to advance research and enter an era of personalised medicine, it will be important to integrate all of these methods in a systems biology approach to fully define the response of a tumour to chemotherapy, which will allow a fully individualised treatment regime to be designed for each patient with the hope of decreasing toxicity and increasing overall response and survival rates.

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