What is the role and impact of molecular markers on treatment decisions in the adjuvant setting of colorectal cancer?

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Despite curative surgery for localized disease, approximately 40% of colorectal cancer patients will eventually relapse. In patients with stage III disease, adjuvant chemotherapy has been shown to lead to definite improvements in survival. The situation for those with stage II disease is more uncertain, with up to 80% thought to be cured by surgery alone. Therefore, there exists a need to identify, pre-treatment, those stage II patients who would benefit from adjuvant chemotherapy and molecular markers may give us important insight into this challenge. Individualized therapy currently is routinely based on clinical observation and drug pharmacokinetics [1] to modulate drug dose and side-effects. Increasingly, however, molecular markers of the tumour and the pharmacogenomic profile of the patient will be taken into account when selecting the appropriate chemotherapy.

In adjuvant colorectal cancer treatment a sufficient level of evidence suggests that DNA indices, angiogenesis indicators and some genetic/biochemical markers identify prognostic differences in patients with early stage colorectal cancer [2]. As is typical in association studies, numerous reports on the prognostic significance of genomic instability have produced only modestly consistent findings. Using meta-analyses it has been shown that high microsatellite instability (MSI+), caused by mutations in the mismatch repair genes leads to improved survival [3], while chromosomal instability (CIN+) is almost certainly associated with a poorer prognosis [4]. Loss of heterozygosity at chromosome 17p or 18q is a common event in colorectal cancer and several studies have focused on the 17p region containing the p53 tumour suppressor gene. p53 has been well studied as both a prognostic factor and a predictor of response to therapy, with conflicting results, perhaps dependent on stage of disease [5]. Mutations in oncogenes such as K-ras have been positively associated with recurrence, poor survival [6] and subsequent metastasis [7], although again these findings have not been consistently replicated in subsequent studies. The angiogenic potential of colorectal cancer has also been found by meta-analysis to impact on recurrence-free and overall survival [8], and suggests biomarkers such as vascular endothelial growth factor (VEGF) expression and microvessel density could be useful future tools for predicting those patients who are at high risk of recurrence.

Evidence is also accumulating that tumour markers specific to 5-fluorouracil (5-FU) could be useful as both prognostic and predictive factors for treatment outcome. Levels of expression of the 5-FU drug target thymidylate synthase (TS) have been widely associated with both prognosis and drug efficacy over the last decade [9, 10]. A meta-analysis of thymidylate synthase expression and prognosis concluded that tumours expressing high levels of TS appear to have a poorer overall survival than tumours with low TS expression [3]. In addition dehydroprymidinase dehydrogenase (DPD) and thymidine phosphorylase (TP) expression have also been shown to play a predictive role in patients receiving adjuvant 5-FU, both independently and in combination [11]. In a multivariate study of TS, TP and DPD, patients with low expression of all three markers had significantly improved survival, but TP did not substantially improve the predictive power of TS and DPD expression for 5-FU-based chemotherapy [12]. Recent microarray studies in 5-FU or oxaliplatin sensitive and resistant cell lines have proposed novel genes which may be important markers of response to treatment [13].

The use of pharmacogenetics in personalized therapy is coming increasingly to the fore. The genetic makeup of a patient plays a major role in governing the response to chemotherapy, affecting the degree of tumour shrinkage and the host toxicity [14]. The study of inherited single nucleotide polymorphisms (SNPs) as biological markers of individual response has the potential to have a great impact on conventional treatment. These SNPs may reduce (or more rarely increase) the function of the translated protein via effects on expression, activity or stability and modulate pharmacokinetic or pharmacodynamic effects of the chemotherapeutic agent. This is the case with irinotecan, and its active metabolite SN-38, the latter of which is conjugated and detoxified by UDP-glucuronosyltransferase (UGT) 1A1 enzyme. Genetic polymorphisms in UGT1A1 affect the interindividual variation in toxicity via decreased detoxification and clearance of the metabolite SN-38 [15]. The first commercial genetic test for identification of potential serious side-effects of irinotecan (via detection of the UGT1A1 *28 polymorphism) was approved by the US Food and Drug Administration (FDA) in 2005. Drug labelling now includes dosing recommendations based on the results of the test. A
similar deficiency in DPD, the enzyme responsible for 5-FU metabolism, is an established indicator of severe toxicity and pharmacogenetic determinants have been investigated. A large number of functional SNPs have been discovered by sequencing the \textit{DPYD} gene in patients exhibiting severe toxicity, with one polymorphism of interest identified at a splice site (IVS14+1G>A) which produces a truncated protein with limited enzymatic activity. However, this polymorphism is relatively rare and does not account for all observed 5-FU toxicity [16]. In addition, about a third of patients carrying this mutation exhibit do not exhibit severe (defined as grade 3 or 4) toxicity after 5-FU treatment. A recent study identified two additional SNPs which, along with the splice site mutation, could form the basis of a genetic test for toxicity. These three SNPs together yield a positive and negative mutation, could form the basis of a genetic test for toxicity. 

Alongside the body of research investigating 5-FU-specific factors, clinical studies of oxaliplatin have frequently incorporated a biomarker component, commonly by examining pharmacogenetic determinants of treatment outcome. Decreased sensitivity to platinum agents has been attributed to diminished cellular drug accumulation, increased intracellular drug detoxification and enhanced DNA repair [21]. Glutathione-S-transferase (GST) has been implicated in several studies on platinum resistance [22] and a number of different polymorphisms within GST enzymes found to correlate with enzyme activity variations [23]. The isoenzyme GSTP1 is thought to be primarily responsible for the detoxification of platinum derivatives and is also the predominant GST in the majority of tumours [24]. Two amino acid changing polymorphisms within GSTP1, Ile105Val and Ala114Val, have recently been shown to predict neuropathy after oxaliplatin treatment [25] as well as the former being implicated in predicting survival [26]. Other common null alleles in GSTM1 and GSTT1 have not been found to be associated with clinical response [27, 28] and their specific role remains uncertain. Since the primary antitumour mechanism of platinum agents is formation of DNA adducts, several polymorphisms in different DNA repair enzymes have been examined for effects on efficacy (reviewed in Kweekel et al. [29]). Proteins of the base and nucleotide excision repair pathways in particular are thought to play a key role and have been well studied in relation to treatment with both cisplatin and oxaliplatin [14]. Relevant genomic polymorphisms have been described in many genes, and preliminary data support the notion that the prediction of response to platinum may be possible based on analysis of certain genotypes. However, out of six commonly studied functional polymorphisms in four repair genes (\textit{ERCC1, ERCC2, ARCC1, XRCC3}) only \textit{ERCC1 Asn118Asn} and \textit{ERCC2 (XPD) Lys751Gln} were consistently associated with overall survival in colorectal cancer [28, 30, 31]. The initial evidence suggests genetic polymorphisms in detoxifying enzymes and DNA repair genes play a small but important role in treatment response to oxaliplatin. There remains a need for further large studies to assess the effect size of these and other novel genetic polymorphisms on the clinical efficacy and toxicity of platinum-based chemotherapy.

Randomized controlled trials investigating prognostic and predictive factors in colorectal cancer are currently under way worldwide. One early such trial was the QUASAR study, designed to test if chemotheraphy prolonged the life of patients with Stage II cancer after surgery [32]. This study showed on average, a small (3–4%) but definite survival benefit after chemotherapy. The question remained, however, whether chemotherapy was beneficial to all patients or whether instead treatment should be more tailored to the patient. Thus a model of cancer recurrence, entitled the ‘Quasar Index’ was developed. This incorporated 12 pathological and 20 biological variables, and by using this index patients who were ‘high risk’ for early recurrence could be identified in advance. The dominant prognostic factors were classical pathological features like T4 stage and evidence of vascular or lymphatic invasion. Of the somatic molecular markers, the only biomarker which entered the multivariate model was the pyrimidine salvage enzyme, deoxouridine triphosphatase, which was a good prognostic variable (Grummet, personal communication). The follow-up study, Quasar II, is currently recruiting patients and is comparing response rates to a new anti-angiogenic agent bevacizumab (Avastin), used in combination with a standard chemotherapy, capcitabine (Xeloda), against capcitabine chemotherapy alone. The trial is also studying known and novel prognostic and predictive markers of response and toxicity, made possible by the collection of a large bio-repository of clinical samples including blood and fresh frozen tissue. A third clinical trial incorporating a biomarker analysis is currently being followed up. The VICTOR study [33] recruited approximately 2500 patients who had undergone apparently curative surgery and chemotherapy/radiotherapy if clinically mandated. Subjects were randomized to receive either the selective COX-2 inhibitor rofecoxib or an identical looking placebo. The primary endpoint of the study was 3-year disease-free survival and a substudy allowed detailed description of the cardiovascular side-effect pattern associated with rofecoxib [34]. Paraffin-embedded tissue has been collected from the primary resection material from this trial, as has blood which allowed extraction of germline DNA from 1000 patients. This has permitted collaboration with the colorectal genetics team led by Ian Tomlinson and Richard Houlston [35–37] and has led to identification of a number of SNPs associated with cancer causation. This work is being extended to detect germline SNPs associated with prognosis and toxicity of chemotherapy. Finally, the SCOT trial, a study of short-course XELOX/OxMdG, also currently recruiting, is another
excellent platform which enables this type of translational research.

In summary, these in-depth studies of tumour biomarkers and pharmacogenetics will enable researchers to better understand the observed individual differences in treatment outcome in the adjuvant setting. Once validated in large-scale randomized clinical trials with adequate statistical power, the selection of patients for personalized medicine using molecular markers may become incorporated into routine clinical practice.

disclosures

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references


2. Hassan AB, Paraskeva C. Colorectal cancer prognosis: is it all mutation, and pharmacogenetics will enable researchers to better understand the observed individual differences in treatment outcome in the adjuvant setting. Once validated in large-scale randomized clinical trials with adequate statistical power, the selection of patients for personalized medicine using molecular markers may become incorporated into routine clinical practice.

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