Genetic testing and surgeon decision

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Colorectal cancer is a highly treatable and often curable disease when localized to the bowel. Traditional pathological staging systems have been useful in predicting the outcome of colorectal cancer, but it is now evident that colorectal cancer is heterogeneous and its natural history strongly correlates with genetic alterations that occur during progression from adenoma to carcinoma to metastatic disease. The goal of many studies is to define a marker, or set of markers, on which therapeutic decisions could be made with greater precision for given individuals. In investigations in which at least 100 patients with locally advanced colon cancer have been studied, those in which monoclonal antibodies to p53 (PAB 1801/DO-7/D0–1) were used have generally demonstrated that mutant or overexpression of p53 is associated with a worse clinical outcome.

Key words: colorectal cancer, pathological, staging

INTRODUCTION

Colorectal cancer is a highly treatable and often curable disease when localized to the bowel. It is the second most frequently diagnosed malignancy and the second most common cause of cancer death in the western world. Although surgical resection alone is potentially curative, local or distant recurrence following surgery is a major problem in many patients and often is the ultimate cause of death. Patients with highest risk of recurrence are advised to receive fluorouracil-based systemic adjuvant chemotherapy which has been shown to be beneficial in several trials. However, the use of chemotherapy in patients with advanced or recurrent metastatic colorectal cancer has been suboptimal despite several decades of active investigation.

Traditional pathological staging systems have been useful in predicting the outcome of colorectal cancer, but is now evident that colorectal cancer is heterogeneous and its natural history strongly correlates with genetic alterations that occur during progression from adenoma to carcinoma to metastatic disease. After over 50 years of experience in oncologic therapeutics development, it is clear that the advancement of cancer therapeutics mandates an improved algorithm to replace the current selection of therapy based on histologic appearance and organ of origin of a particular cancer. Cancer is a genetic disease, and it is within the genes and their ultimate expression that lie the keys to the development of successful therapeutics. Numerous predictive and prognostic molecular diagnostic markers have been investigated in patients with colorectal cancer, with the goal of aiding in the selection of the best available therapeutic strategy or prognosticating the probable longevity of a particular patient. The goal of many studies is to define a marker, or set of markers, on which therapeutic decisions could be made with greater precision for given individuals. Thymidylate synthase (TS), p53, measures of proliferative rate (i.e., Ki-67 expression), and the status of mismatch repair (MMR) complex components, the last one evaluated through microsatellite instability (MSI) status characterization, are among the most heavily investigated parameters. These markers have been demonstrated in a number of studies to have potential value in defining populations of individuals who either may or may not benefit from the use of adjuvant chemotherapy. It seems also possible that these markers may serve as an adjunct for the prognostication of natural history for those likely to develop advanced colon carcinoma.

Of all potential markers that may have prognostic or predictive value for patients with colon cancer, p53 has been the most investigated. Mutations in p53 have been found to occur in 40% to 60% of patients with colon cancer. Preclinical investigations have demonstrated that mutant p53 renders malignant cells less sensitive to most chemotherapeutic agents, with the exception of the taxanes, which seem to be indifferent to p53 status. Given the logistical difficulties and resources associated with direct sequencing of the p53 gene, most investigations have
used immunohistochemistry as a means of detecting mutant p53, with the assumption that overexpression of p53 is often associated with a mutation, while the lack of expression is generally indicative of wild-type p53. This assumption has been valid in approximately 60% to 80% of instances in which mutational analysis and p53 detection by immunohistochemistry have been compared. Whereas numerous investigations have been performed addressing the possible prognostic and/or predictive value of p53 in patients with colon cancer, the majority have been performed in small numbers of individuals and thus suffer from limited statistical power. This issue is further compounded by the availability and use of multiple antibodies, which have varying levels of sensitivity and capacity for detecting mutant protein. In investigations in which at least 100 patients with locally advanced colon cancer have been studied, those in which monoclonal antibodies to p53 (PAB 1801/DO-7/D0–1) were used have generally demonstrated that mutant or overexpression of p53 is associated with a worse clinical outcome. However, this association has not been a constant finding; several investigations have found either the opposite or no association between the expression of p53 and clinical outcome. Thus, while the general impression is that the overexpression of p53 is associated with a less-favorable clinical outcome for patients with locally advanced colon cancer, investigations that demonstrate contrary associations indicate that the role of p53 as a prognostic marker requires additional investigation.

Flow cytometry has been the technology most commonly used to measure cell cycling activity in patients with colon cancer. Several investigations have found that a high S-phase fraction (20%) is associated with a greater probability of recurrence and diminished survival, although this has not been universally observed. Ki-67 is expressed in cells actively engaged in the cell cycle and has also been used as a measure of proliferation in this patient population. Unfortunately, most of these studies are relatively small and have not demonstrated a consistent association with clinical outcome.

TS is the primary intracellular target for the fluoropyrimidine class of chemotherapeutic agents that includes fluorouracil (FU) and capecitabine and several new antifolate agents under clinical development. This enzyme is responsible for the provision of thymidine required for DNA synthesis and repair. Both preclinical and clinical investigations have demonstrated the importance of intracellular TS levels as a determinant of sensitivity to FU, and multiple clinical investigations have demonstrated an improved response to fluoropyrimidine-containing regimens in patients with low levels of TS in their cancers compared with the response in patients whose cancers overexpress TS.

A recent investigation adds, given the large number of patients analyzed, relevant supportive data to the value of TS, K-67 and p53 as prognostic markers. The study concerns 706 colorectal cancer patients, including 275 patients with Dukes B and C treated with surgery alone and 431 treated with surgery followed by weekly bolus of 5-fluorouracil plus leucovorin therapy. The data obtained demonstrated that TS intensity, p53 positivity, and low levels of Ki-67 nuclear staining were each significantly associated with a poor 5-year relapse free survival (RFS) and/or over all survival (OS). A high level of Ki-67 nuclear staining, that is, greater than 40% of cells positive, was associated with relative risk of 0.76 and 0.62 for 5-year RFS and OS, respectively. A high level of TS staining intensity was associated with a relative risk of approximately 1.5 for both RFS and OS. Positive p53 associated with a relative risk of approximately 1.5 for RFS and 1.2 (not statistically significant) for OS. In the multivariate analysis that included treatment and Dukes' stage, high TS scores and smaller values of Ki-67 both correlated with a significantly poorer OS, while high TS scores and p53 positivity were each associated with a significantly poorer RFS. Smaller values of Ki-67 were associated with a poorer RFS. The results of the investigation confirmed the data reported in literature concerning an interaction between TS staining intensity and clinical outcome. Interaction with treatment was not observed for any of the markers analyzed therefore none of the markers could be used to identify the patients who would obtain greater or lesser benefit from the use of adjuvant chemotherapy.

The same authors have investigated the utility of quantifying thymidine synthase (TS) in the primary tumor as a surrogate for metastatic disease sites to predict the likelihood of response and outcome to fluorouracil (FU) treatment in patients with metastatic colorectal cancer. Their results showed that the level and extent of TS protein expression in the primary tumor neither correlate with overall survival in patients with metastatic or recurrent colorectal cancer nor aid in predicting response to FU in a metastatic disease site.

As concerning microsatellite instability status, it is well known that colorectal cancers develop as a consequence of genomic instability. One form, chromosomal instability, is the genetic reason for tumor formation in approximately 80%–85% of colorectal cancers. These colorectal cancers have allelic loss of certain tumor suppressor genes after an initial allele has been mutated (e.g., adenomatous polyposis coli or P53), as originally predicted by Fearon and Vogelstein. A second form of genomic instability, called microsatellite instability (MSI), is involved in the genesis of 15%–20% of colorectal cancers. In the last case tumors have multiple errors in repetitive DNA sequences, termed microsatellites, throughout their genome. This is the consequence of a failure to edit errors made during DNA replication which occurs when the DNA mismatch repair (MMR) system is defective due to mutations more frequently involving MLH1, MSH2, MSH3 and MSH6 genes.

In addition in most sporadic MSI colorectal cancers, epigene hypermethylation of the hMLH1 MMR gene occurs with silencing gene transcription of hMLH1, inactivating the DNA MMR and allowing the occurrence of MSI. High-frequency microsatellite instability phenotype includes a correlation with the tumor’s location in the
proximal (right) colon and an inverse relation to allelic loss. Additionally, MSI colorectal tumors tend to be diploid, tend to possess a mucinous histology, and have a lymphoid reaction surrounding the tumor. Chromosomally unstable colorectal tumors, for example, are aneuploid and have an overall poorer survival compared with MSI tumors.

Work with in vitro cell models of MSI has indicated a difference in response to chemotherapeutic agents that might be extended to the clinical situation. The alkylator N-methyl-N'-nitro-N-nitrosoguanidine makes the principle adduct 8'-methylguanine, which can be recognized by cells with a competent MMR (i.e., cells without MSI) to effect arrest at the G2 phase of the cell cycle and prohibit growth. Cells with MSI did not respond to the alkylating agent and continued to grow with active cell cycling. Similarly, the nucleotide analogue 6-thioguanine, when incorporated into MMR-competent cells, arrested cells in the G2 phase of the cell cycle, a feature absent in MSI cells incorporated with 6-thioguanine. The MMR proteins recognize cis-platinum adducts. Finally, in vitro work with 5-FU indicated that colon cancer cells with MSI might not respond readily to this agent. Some 5-FU was incorporated into DNA, showing the possibility of recognition by the DNA MMR system. Cells that were proficient in MMR were killed by treatment with 5-FU, compared with the sparing of MSI colon cancer cells. Additionally, re-expression of hMLH1 in an MSI cell line with hypermethylated hMLH1 overcame resistance to treatment with 5-FU.

The type of genomic instability within a colorectal cancer might dictate how the patient responds to 5-FU-based chemotherapy. Very recently two robust studies, including 774 affected individuals, evaluated the efficacy of 5-FU in the treatment of patients with MSI colorectal cancers. Both the investigations demonstrated that whereas patients whose tumors are non-MSI-high derive a benefit from 5-FU-based chemotherapy, with an improvement in survival, patients with MSI-high tumors do not.

Tailoring 5-FU treatment on the basis of the biology of a patient's tumor, rather than just the stage, may become more frequent as we come to understand the clinical differences among the genetic causes for colorectal cancer development.

The data so far collected show that basic laboratory findings may indeed have a clinical application.

What steps are necessary to speed the progress of moving prognostic/predictive markers into clinical practice? First, large, simple trials, with reduced eligibility criteria and data collection requirements, would allow larger trials to be feasibly conducted, thus enriching our databases. Second, careful consideration needs to be given to mandating tissue collection on clinical trials, to eliminate the potential bias introduced by subset analyses. Third, the evaluation of markers needs to be considered at the design stage of a trial, and consideration needs to be given to designing trials specifically aimed at marker questions.

However the development of the microarray technologies promises a better characterization of the different types of cancers and creation of a new cancer taxonomy, that will certainly lead to a revolution in cancer therapeutics.

REFERENCES


