The use of gene expression profiling in the personalization of colorectal cancer treatment

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Colorectal cancer (CRC) is the third most common cancer in Canada. This year in Ontario, it is estimated that 8700 people will be diagnosed with CRC and 3450 people will die of the disease. Due to the high mortality rate of CRC, screening programmes have been designed to screen the population for CRC prior to the onset of symptoms. The high burden of disease CRC causes has also made the field of CRC screening, diagnosis and treatment a prime area for the development of novel medical technology. Historically, these technologies have been limited to the fields of biochemical testing of stool and fiber optic visualization of the colon using the colonoscope. A new and exciting field for technology development is the application of molecular genetics in the profiling of colorectal tumours. Genetic profiling will allow clinicians to develop a personalized treatment plan for the administration of chemotherapeutic agents and biologics in the treatment of CRC. Personalized medicine has the potential to improve outcomes by selecting the chemotherapeutic agent that has the best likelihood of effectiveness for a given patient’s specific tumour. In order for this practice to become the standard of care, links between genetic profiles and chemotherapeutic effectiveness must be known and technologies for analysing the relevant gene profiles must be developed. The application of this technology to stage II CRC in particular is an active field of research.

Currently, the five year survival rate for stage I CRC is approximately 93%. This survival rate falls to approximately 80% for patients with stage II disease. The effectiveness of adjuvant chemotherapy for stage II CRC disease is currently an area of debate in the field of oncology. Moertel et al. conducted a retrospective study looking at patients with stage II CRC to identify clinical characteristics that are predictive of recurrence of disease in order to identify patients who might benefit from adjuvant chemotherapeutic treatment. These characteristics include T4 lesions, bowel obstruction and tumour perforation. Patients with high risk stage II disease, identified using these characteristics, are often treated with adjuvant chemotherapy, despite the lack of validation with a randomized clinical trial. An analysis of the American SEER-Medicare database--one of the largest medical databases in the United States--has demonstrated that patients with stage II CRC who received adjuvant fluorouracil based chemotherapy did not have improved outcomes with either high risk or low risk disease. Conversely, the QUASAR study has shown that the standard chemotherapy regimen of fluorouracil plus leucovorin produces a 3% survival benefit in stage II CRC; however, the toxicity of the treatment causes death in 0.5% of patients. To make matters more complicated, the addition of oxaliplatin to a chemotherapeutic regimen of fluorouracil and leucovorin improves the outcome in stage III CRC but does not improve the 6 year survival outcomes of stage II CRC. This surprising finding highlights the need for improving the clinical criteria for selecting stage II CRC patients who will benefit from adjuvant chemotherapeutic treatment. The findings in this paper also suggest that CRC is not necessarily a stepwise progression from stage II to stage III disease and demonstrates the need for further research into the molecular biology of stage II disease.

Despite the criteria for high risk stage II CRC outlined above and the limitations of these criteria, the morbidity associated with treatment for stage II CRC could be greatly reduced if physicians were able to further delineate which patients could benefit from adjuvant treatment. The molecular genetic profiling of a patient’s colorectal tumour could potentially yield this information by analysing for the expression of genes which are known to respond better to adjuvant chemotherapy. While this approach is very exciting and holds great promise, physicians and researchers need to make sense of the extensive data that can be generated using a genomics approach. Knowing the expression levels of all the genes in the CRC tumour is not a practical approach to deciding treatment options for the disease. Several research groups have addressed this challenge by selecting groups of genes to profile in an effort to determine which genes in stage II CRC’s are clinically significant for recurrence.

An interesting product that is beginning to become available in the clinic is the Oncotype Dx Colon Cancer 12 gene assay. This assay measures the expression levels of seven cancer related genes (Stromal Group: BGN, FAP, INHBA; Cell Cycle Group: MYC, MK167, MYBL2; Early Response Group: GADD458) and normalizes these genes to a group of reference genes that have very little variability in expression (ATP5E, GPX1, PGK1, VDAC2, UBB). The seven cancer related genes were selected based on the results of a series of development studies which included four independent cohorts for a total of over 1800 patients. Starting with a panel of 761 genes known to be associated with CRC, the seven genes most associated with recurrence risk and differential benefit with adjuvant chemotherapy in stage II CRC were selected to be used on the Oncotype Dx 12 gene assay. Tissue samples for use in this particular assay are fixed in formalin, a significant technical advancement over other assays since it means that pathology samples and gene expression profiling can be obtained from one tumour sample. It also allows gene expression profiling to be conducted on previous biopsies or tumour resections. Briefly, RNA is extracted from the formalin fixed tumour and quantified to determine the amount of RNA present in the sample. The RNA is then reverse transcribed to complementary DNA using a gene specific primer for each assay gene. The expression levels of the 12 genes are then measured using quantitative polymerase chain reactions (PCR) (see Figure 1 for a schematic of the assay). After the molecular biologic analysis is completed, a recurrence score between 0 and 100 is then assigned based on an algorithm outlined by Clark-Langone et al.. A low score (≤30) corresponds to a 3 year recurrence risk of 8%, an intermediate score (31-40) has a 3 year recurrence risk of 11% and a high score (≥41) has a 3 year recurrence risk of 25%.
This algorithm takes the combined expression values from each of the gene groups (Stromal, Cell Cycle and Early Response), allowing for co-expression, and combines these values with varying weights to create an overall recurrence score, an individualized risk estimate for colon cancer recurrence. While initial results based on a retrospective analysis using the QUASAR trial do show an association between the recurrence risk score and prognosis, the predictive value of the recurrence score for treatment benefit has not yet been shown. Further trials are underway to determine the full clinical effectiveness of the Oncotype Dx Colon Cancer Assay.  

Other similar products on the market include the Coloprint 18 gene assay; however, this assay is of limited utility since it only makes use of fresh frozen tumour samples. The Oncotype Dx Colon Cancer Assay uses formalin fixed tissues allowing for the flexibility to also perform pathological analysis of the same tumour sample. While these emerging technologies are not likely to replace the role of pathological evaluation of tumours in the diagnosis and staging of colorectal cancers within our lifetime, they may have the ability to complement current practices by providing more detailed information on which treatment options are best for a particular patient. Despite their promise, several questions need to be addressed regarding their utility. Since a patient has many CRC tumours and not all tumours have an identical genotype, will the assays be valid at selecting those patients who will benefit from adjuvant chemotherapy? Moreover, since the genotype of a CRC tumour changes during the course of treatment due to the development of drug resistance, will the assay be a worthwhile clinical tool in the treatment of the ever evolving stage II CRC tumour? Also, how much of a survival advantage will adjuvant therapy provide and will the assay be cost-effective when analyzed using QALY? Despite these questions, the use of molecular

**Figure 1:** A schematic diagram illustrating the Oncotype Dx Colon Cancer Assay. A colorectal tumour is fixed in formalin, sent for pathological analysis and used concurrently in the Oncotype Dx assay. RNA is extracted from the tumour and is quantified using fluorescent dyes. A known amount of RNA is added to a reverse transcription reaction. The RNA is reverse transcribed to complementary DNA (cDNA) using primers specific for each of the 12 genes of interest. Quantitative polymerase chain reaction (qPCR) is performed using the cDNA and fluorescent probes. The probes are specific for each gene of interest and have a fluorescent molecule (star) bonded to one end and a quencher (spiral) that stops the fluorescence bonded to the opposite end. During the PCR reaction the Taq polymerase enzyme cleaves the probe thus, releasing the fluorescent molecule from the quencher. The amount of fluorescent signal in the reaction increases and is measured. The expression levels of the genes of interest are normalized against the reference genes using the Oncotype Dx algorithm.
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genetics tools in personalized cancer treatment is sure to become a reality over the course of our medical careers.

REFERENCES


