Genomic profiling of colorectal cancer

Vicky M. Coyle, Patrick G. Johnston

Centre for Cancer Research and Cell Biology, Queens University Belfast, Belfast, Northern Ireland

Introduction

Novel genomic technologies such as microarray profiling have revolutionised the study of cancer development and progression in a range of malignancies, including colorectal cancer. Studies utilising these methodologies have provided insights into the genomic alterations that potentially underpin the processes of carcinogenesis, tumour growth and metastasis, and enabled the identification of gene signatures predictive of tumour recurrence or response to a given treatment modality. However, whilst the initial results have shown great promise, it has become apparent that several challenges will have to be overcome to implement this technology into clinical practice.

Genomic technologies

A number of high throughput genomic technologies have been used as research tools in colorectal cancer, including single nucleotide polymorphism (SNP) analysis, array comparative genomic hybridisation (CGH) and microarray-based gene expression profiling. Each approach has potential clinical applications, for example, the identification of an association between a particular genetic polymorphism and drug toxicity, such as the increased toxicity following irinotecan therapy observed in patients with the UGT1A1∗28 polymorphism [1].

Clinical application of microarrays in colorectal cancer

In general terms, microarray studies tend to have one of three main objectives: class discovery, class comparison and class prediction [2].

Class discovery

Class discovery involves identifying trends within the data, for example, the identification of molecular subtypes of tumours with similar clinicopathological characteristics.

Class comparison

Class comparison involves the identification of genes that are differentially expressed between samples belonging to pre-defined classes [3]. In colorectal cancer microarray technology has been used to identify differentially expressed genes between normal mucosa and colorectal tumour [4] and between colorectal tumours of different stage [5]. There is some commonality in the genes identified in these studies: a recent meta-analysis of 25 studies identified some genes that were differentially expressed between carcinoma, adenoma and normal tissue with a statistically significant frequency [6], suggesting that further investigation of these genes might yield insights into tumour progression.

A recent study has used gene expression profiling data to identify genes differentially expressed in responding and non-responding patients receiving cetuximab monotherapy, with the EGFR ligands epiregulin and amphiregulin included in the significantly differentially expressed genes. Additionally, an association between high levels of expression of these genes and significantly increased progression-free survival was found [7].

Class prediction

Class prediction is similar to class discovery except the objective is to construct a classifier, a mathematical function that outputs a prediction of the class of an unknown sample [8]. Class prediction studies in colorectal cancer have largely focused on prognosis prediction in early stage disease.

Prognosis prediction

Microarray profiling has been used to identify predictors of recurrence in Stage II and III colorectal cancer, with one such early study reporting the identification of a 23-gene signature associated with disease

recurrence in Dukes B cancers, which demonstrated a predictive accuracy of 78% [9].

A further study identified a predictive classifier that performed well when assessed by cross-validation within the same dataset (predictive accuracy 90%). This high level of predictive ability was maintained when the classifier was tested in an independent validation set of patients using a different microarray platform [10].

Barrier and colleagues have taken two different approaches in using this technology for prognosis prediction in early stage CRC. Firstly, predictive classifiers were built using both non-neoplastic mucosal (NM) and neoplastic mucosal (M) samples from each patient and the performance compared: the NM predictive classifier performed better than the M classifier (predictive accuracies of 83% and 78% respectively), perhaps due to the increased homogeneity of the mucosal samples [11]. Secondly, this group investigated the effect of changes in training and test set size on the performance of predictive classifiers generated from the same microarray dataset, demonstrating variability in the performance of the resultant classifiers, with an average predictive accuracy of 76.3% overall. As might be expected, classifiers constructed from larger training sets demonstrated better predictive ability, although multiple predictors of different content demonstrated similar performance [12].

Treatment response prediction

Clinical studies evaluating microarray-based treatment response prediction in colorectal cancer are limited. A small number of studies have used this approach to build predictors of response to chemoradiotherapy in locally advanced rectal cancer. Ghadimi and colleagues performed gene expression profiling of pre-treatment rectal biopsies from patients who received chemoradiotherapy prior to surgical resection. Response classification was based on a reduction in tumour 'T' stage and pathological regression. A 39-gene predictor was generated which performed with 83% accuracy, as assessed by cross-validation, and with 86% accuracy when used to predict response in a smaller data set profiled on a different array platform [13].

A similar study by Kim and colleagues identified a 95-gene predictor of chemoradiotherapy response that performed with a predictive accuracy of 84% as assessed by cross-validation; this predictor performed with an accuracy of 87% in a small test set.

Only one study has described a chemotherapy response predictor in the setting of advanced colorectal cancer. Del Rio and colleagues performed gene expression profiling of pre-treatment tumour samples from 21 patients with metastatic colorectal carcinoma who received irinotecan/5-FU chemotherapy (FOLFIRI) and who were classified as responders or non-responders based on radiological response. A 14-gene predictor was identified that performed well when assessed by cross-validation (predictive accuracy 95%). Additionally, using a multiple random training-validation strategy, predictors built using these 14 genes generally performed well, with a misclassification rate of at most 25.6% for small training sets and only 7.5% for training sets with 13 or more samples [14].

Limitations of clinical microarray studies

The gene expression profiling studies investigating prognosis prediction and treatment response prediction in colorectal cancer to date have been, in general, small studies with heterogenous patient populations, variations in the microarray technology and data analysis methods used and variations in study endpoints. Consequently, the predictive classifiers identified in these studies would require validation in larger studies using independent patient cohorts that have strict inclusion criteria and pre-defined study endpoints before their use in routine clinical practice could be considered.

Conclusion

The recent advances in the management of colorectal cancer have been paralleled by exciting developments in genomic technologies, and a number of researchers have utilised these technologies; in particular, microarray-based gene expression profiling, to further our understanding of colorectal cancer biology, tumour progression and response to therapy. Combining such an approach with novel therapeutic agents will only serve to bring the goal of individualised treatment for patients closer.

Conflict of interest statement

Main form of employment

Dean, School of Medicine & Dentistry and Director, Institute of Health Sciences, Queen's University Belfast; Professor of Oncology, Queen's University Belfast

Directorships

Almac Diagnostics (Founding Director and shareholder)

Shareholdings in Companies

Fusion Antibodies; GlaxoSmithKline

Consultancy work to Biopharmaceutical Industry

Over the last several years I have acted as a Consultant to the following companies: AstraZeneca; Pfizer; Roche Pharmaceuticals; Merck; Amgen; Bristol-Myers Squibb (BMS).

These consultancies have consisted of providing advice in relationship to aspects of drug development from Phase I–II clinical trials and also in the area of pharmacogenomics.

Patents (either filed or in process)

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(2) **Johnston, PG,** Fisher, E, Allegra, CJ. Thymidylate synthase antibodies as predictors of outcome and response in gastrointestinal cancers. Patent Number 1389295,84
(3) **Johnston PG**, Allegra CJ. Thymidylate synthase antibodies as predictors of outcome and response to chemotherapy in breast cancer. Patent Number 35/672309.
(4) **Johnston PG,** Allegra CJ. Thymidylate synthase antibodies as predictors of outcome and response to chemotherapy in head and neck Cancer. Patent Number 34/709891.
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(6) **Johnston PG,** Longley DL. Fas-related peptides as anticancer targets. Patent Filed May 2002.
(7) Mulligan KA, **Johnston PG,** McCormick D. Use of MQ1 antibody and MQ1 protein antisense oligonucleptodes in cancer diagnosis and treatment. Patent Filed January 2003.
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