LEADING ARTICLE

Colorectal cancer prognosis: is it all mutation, mutation, mutation?

A B Hassan, C Paraskeva

Gut 2005;**54**:1209–1211. doi: 10.1136/gut.2005.070946

For the 500 000 new cases of colorectal cancer in the world each year, identification of patients with a worse prognosis and those who are more likely to respond to treatment is a challenge. There is an increasing body of evidence correlating genetic mutations with outcome in tumours derived from human colorectal cancer cohorts. K-ras, but not p53 or APC, mutations appear to be associated with poorer overall survival in colorectal cancer patients.

For the 500 000 new cases of colorectal cancer in the world this year, staging of their disease and subsequent treatment will be almost entirely based on a histopathological classification originated by Cuthbert Duke in the 1920s. While there is nothing immediately wrong with this classification and its derivatives, in particular because it has been universally adopted, it is clear that significant heterogeneity exists between patient outcomes with otherwise apparently identical pathological staging. The immediate challenge faced by the clinical community is to identify patients with a worse prognosis and those who are more likely to respond to treatments, such as surgical resection, radiotherapy, chemotherapy, and molecular therapy.

Patient selection is also now more of an important issue for a series of scientifically based reasons. These include identification of patients at high risk from colorectal cancer prior to screening and prevention programmes, those with familial predisposition (35% of total), including mutation screening of key genes (2–6% of total), and more recently in systemic treatment, as empirically applied treatments including cytotoxic chemotherapy are now being challenged by new molecular therapies. Some of the new molecules are currently administered empirically, as the co-development of molecular testing and tailored treatments has not kept pace with the enthusiasm of the immediate therapeutic testing.[1 2] In time, this situation will reverse as the costs of treatments increase, and we should expect a series of predictive tests to be developed that facilitate selection based on currently available targeted therapy. In situations where targeted molecular therapy has been developed with a validated molecular diagnostic test (for example, breast (Her2), chronic myeloid leukaemia (BCR-ABL), gastrointestinal stromal tumours (C-KIT), and lung cancer (EGFR)), there have been dramatic improvements in disease management that far exceed anything that has been achieved with an empirical approach. What is critical is that the selectivity and potency of these therapies exploits mutation in the tumour cells, with amplification of Her2, translocation in BCR-ABL, and mutation of receptors C-Kit and EGFR. Although an expensive challenge, the cancer field is rightly continuing to pursue the identification and evaluation of common human mutations in cancer, as evident by the Cancer Genome project.[3] This activity will no doubt drive the development of specific therapeutic interventions to specific mutated targets. Despite the success to date in other cancers, this whole activity could be an expensive mistake if a combination of mutation complexity, aneuploidy, and epigenetic alterations in tumour cells ultimately determine prognosis and response to therapy, irrespective of targeted therapy to what are presumed to be key mutations.

> ''Although an expensive challenge, the cancer field is rightly continuing to pursue the identification and evaluation of common human mutations in cancer, as evident by the Cancer Genome project''

So what of mutations in colorectal cancer? The paper by the Dundee group[4] in this issue of *Gut* contributes to an increasing body of evidence correlating mutation with outcome in tumours derived from human colorectal cancer cohorts ***(see page 1283)***. Before examining their findings, it is important to recognise that mutational analysis and genetic models have already identified the key pathways critical for initiation and progression of colorectal cancer. Deregulation of the *Wnt* signalling pathway, by either loss of function or gain of function mutations of adenomatous polyposis coli (APC) and β-catenin, respectively, in both hereditary and sporadic colorectal adenoma, establish the principal initiation pathway.[5] Although specific mutational hotspots occur that account for attenuated inherited phenotypes, most sporadic mutations of APC occur in a mutational cluster.[6 7] The discriminating power and clinical utility of these mutations have proven to be less impressive, even though this pathway remains a critical therapeutic target.[8] Genetic modifiers of the number, differentiation, and progression of

See end of article for authors' affiliations

Correspondence to: Professor A B Hassan, Department of Cellular and Molecular Medicine (formerly Pathology and Microbiology), School of Medical Sciences, University of Bristol, Bristol BS8 1TD, UK; Bass.Hassan@Bristol.ac.uk

Revised version received 24 May 2005
Accepted for publication 26 May 2005

Abbreviations: K-ras, Kirsten-ras; APC, adenomatous polyposis coli

colorectal adenoma can either derive from the host or from coexisting and selected mutations in the tumour. Dissecting these components has been problematic, mainly because a large number of variables exist (for example, variable penetrance, gain and loss of function mutations in each gene, and synergistic effects between modifiers that coexist in both tumour and stromal cell populations). Although these limitations are being circumvented using larger patient numbers with high throughput technologies, there will have to be a practical outcome that will essentially distil down this information to a fewer number of key mutations, and potentially to where we have started from, a classification based on morphology and identification of a subpopulation of localised cells that essentially determine overall outcome (for example, those at the micro-invasive front of the tumour).

''K-ras mutations, but not p53 or APC mutations, were associated with poorer overall survival, even when correcting for Dukes' stage, age, and sex''

The Dundee group prospectively characterised mutations in three genes implicated in colorectal cancer (*APC*, *p53*, *Kirsten-ras (K-ras)*) and extended a previous study.[9] They observed that the mutation frequencies were variable between genes (*APC* = 56%, *p53* = 61%, *K-ras* = 27%), and when they controlled for expected frequencies, p53 and K-ras genes rarely coexisted (observed 2% versus expected 16%), even though the reduction in expected frequency of combined p53 and K-ras appears to have been lost when mutations of APC coexisted in addition. Conlin and colleagues[4] have also now correlated these mutations with survival in colorectal cancer. In a relatively small cohort of 107 patients, the significant additional findings were that K-ras mutations, but not p53 and APC mutations, were associated with poorer overall survival, even when correcting for Dukes' stage, age, and sex. The surprise is that the magnitude of this effect for a single gene appears large in this cohort relative to the influence of Dukes' stage alone, but on closer analysis, the frequency of mutations of K-ras was highest overall in more advanced disease. A similar examination of K-ras mutations in a larger cohort also showed association of the glycine codon 12 to valine mutation with high risk disease.[10] Moreover, similar studies examining p53, chromosomal loss, and microsatellite instability also correlate with survival, with a subgroup recognised that neither falls within a chromosomal nor microsatellite unstable groups.[11 12]

How should we view this information? Clearly, some of the data concern small cohorts, and the magnitude of the effects may be lost in larger studies. Moreover, the complexity of this analysis is complicated because mutations in some genes do not always generate the same phenotypic outcomes. For example, for p53, recent data implicate specific mutations of p53 with colorectal cancer outcome, although there does not appear to be an overall correlation, as Conlin *et al* have suggested.[13–15] Ultimately, mutations and chromosomal abnormalities that are both easier to detect and at high frequencies will be selected first, simply because of the eventual contribution to statistical outcome.[16 17] Some would also argue that a focus solely on mutation would distract from the potential contribution of epigenetic modification of modifier gene expression, which we know is also commonly detected in colorectal cancer.

''Ultimately, mutations and chromosomal abnormalities that are both easier to detect and at high frequencies will be selected first, simply because of the eventual contribution to statistical outcome''

So, are we making too many assumptions about the complexity of the task ahead? Probably not when it comes to deriving useful information, because studies such as Conlin *et al* highlight this fact in terms of specific and common gene mutational frequency in colorectal cancer tissue derived from patients. In addition, the few data there are, are also being immediately assembled with Dukes' stage information, site, and ploidy into an increasing number of subgroups, despite the relatively small cohorts examined.[18] The complexity is very high and no one should underestimate the difficulty. Not surprisingly, there is a need for newer approaches, perhaps to assemble mutations into various pathways, which are best exemplified by the six hallmarks proposed by Hanahan and Weinberg,[19] and to then analyse what intermediate surrogate markers may be used to subdivide and target mutation testing. This may not mean an initial screen for all the potential mutually exclusive mutations along one pathway, but a ''readout'' of its overall activity based on histological, proteomic, or expression profiling.[20–22] The goal of the latter must be to ultimately apply specific interventions, and to test the usefulness of the mutation in the overall context of prevention, diagnosis, prognosis, and therapy. In this case, mutations, and epigenetic and expression alterations are then judged by clinical utility.

ACKNOWLEDGEMENTS

We acknowledge research funding support from Cancer Research UK.

Authors' affiliations

A B Hassan, C Paraskeva, Department of Cellular and Molecular Medicine (formerly Pathology and Microbiology), School of Medical Sciences, University of Bristol, UK

Conflict of interest: None declared.

REFERENCES

1 **Cunningham D**, Humblet Y, Siena S, *et al.* Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004;**351**:337–45.
2 **Hurwitz H**, Fehrenbacher L, Novotny W, *et al.* Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;**350**:2335–42.
3 **Gabor Miklos GL**. The Human Cancer Genome Project—one more misstep in the war on cancer. *Nat Biotechnol* 2005;**23**:535–7.
4 **Conlin A**, Smith G, Carey FA, *et al.* The prognostic significance of K-ras, p53, and APC mutations in colorectal carcinoma. *Gut* 2005;**54**:1283–6.
5 **Reya T**, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005;**434**:843–50.
6 **Crabtree MD**, Tomlinson IP, Hodgson SV, *et al.* Explaining variation in familial adenomatous polyposis: relationship between genotype and phenotype and evidence for modifier genes. *Gut* 2002;**51**:420–3.
7 **de la Chapelle A**. Genetic predisposition to colorectal cancer. *Nat Rev Cancer* 2004;**4**:769–80.
8 **Lepourcelet M**, Chen YN, France DS, *et al.* Small-molecule antagonists of the oncogenic Tcf/beta-catenin protein complex. *Cancer Cell* 2004;**5**:91–102.
9 **Smith G**, Carey FA, Beattie J, *et al.* Mutations in APC, Kirsten-ras, and p53—alternative genetic pathways to colorectal cancer. *Proc Natl Acad Sci U S A* 2002;**99**:9433–8.
10 **Andreyev HJ**, Norman AR, Cunningham D, *et al.* Kirsten ras mutations in patients with colorectal cancer: the 'RASCAL II' study. *Br J Cancer* 2001;**85**:692–6.
11 **Hawkins NJ**, Tomlinson I, Meagher A, *et al.* Microsatellite-stable diploid carcinoma: a biologically distinct and aggressive subset of sporadic colorectal cancer. *Br J Cancer* 2001;**84**:232–6.
12 **Georgiades IB**, Curtis LJ, Morris RM, *et al.* Heterogeneity studies identify a subset of sporadic colorectal cancers without evidence for chromosomal or microsatellite instability. *Oncogene* 1999;**18**:7933–40.
13 **Diep CB**, Thorstensen L, Meling GI, *et al.* Genetic tumor markers with prognostic impact in Dukes' stages B and C colorectal cancer patients. *J Clin Oncol* 2003;**21**:820–9.
14 **Russo A**, Migliavacca M, Zanna I, *et al.* p53 mutations in L3-loop zinc-binding domain, DNA-ploidy, and S phase fraction are independent prognostic indicators in colorectal cancer: a prospective study with a five-year follow-up. *Cancer Epidemiol Biomarkers Prev* 2002;**11**:1322–31.

15 **Forslund A**, Lonnroth C, Andersson M, *et al.* Mutations and allelic loss of p53 in primary tumor DNA from potentially cured patients with colorectal carcinoma. *J Clin Oncol* 2001;**19**:2829–36.
16 **Sieber OM**, Heinimann K, Tomlinson IP. Genomic instability—the engine of tumorigenesis? *Nat Rev Cancer* 2003;**3**:701–8.
17 **Vendrell E**, Morales C, Risques RA, *et al.* Genomic determinants of prognosis in colorectal cancer. *Cancer Lett* 2005;**221**:1–9.
18 **Risques RA**, Moreno V, Ribas M, *et al.* Genetic pathways and genome-wide determinants of clinical outcome in colorectal cancer. *Cancer Res* 2003;**63**:7206–14.
19 **Hanahan D**, Weinberg RA. The hallmarks of cancer. *Cell* 2000;**100**:57–70.
20 **Nishizuka S**, Charboneau L, Young L, *et al.* Proteomic profiling of the NCI-60 cancer cell lines using new high-density reverse-phase lysate microarrays. *Proc Natl Acad Sci U S A* 2003;**100**:14229–34.
21 **Nishizuka S**, Chen ST, Gwadry FG, *et al.* Diagnostic markers that distinguish colon and ovarian adenocarcinomas: identification by genomic, proteomic, and tissue array profiling. *Cancer Res* 2003;**63**:5243–50.
22 **Kimura ET**, Nikiforova MN, Zhu Z, *et al.* High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res* 2003;**63**:1454–7.