Molecular testing of gastrointestinal tumours

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Abstract

Cancers of the gastrointestinal tract are a major cause of morbidity and mortality globally, and not surprisingly, there has been intense interest in identifying prognostic and predictive markers for new targeted therapies. The cancers of the gastrointestinal tract are unique in exemplifying in microcosm the axioms of molecular pathology: that amongst the various organs of the gastrointestinal tract, different molecular markers have different implications; that of the innumerable molecular alterations identified in these cancers, only an extremely small minority have proven clinical relevance; and perhaps most importantly, that molecular pathology practitioners must be prudent in maximising the use of small tissue samples to garner clinically important information. Here, we review the clinically-validated molecular targets in carcinomas of the oesophagus, stomach, colorectum and anal canal, and in gastrointestinal stromal tumours, and discuss a handful of markers which are likely to be of use in the future.

Keywords anus neoplasms; colorectal neoplasms; DNA mismatch repair; gastrointestinal stromal tumours; hereditary non-polyposis; immunotherapy; molecular; oesophageal neoplasms; pathology; stomach neoplasms

Introduction

There has been a recent shift in paradigms within histopathology: no longer is the classification of a tumour dependent purely on its appearance and expression of particular antigens. Rather, there is an increasing — and increasingly rapid — shift towards using molecular criteria for determination of a given tumour's prognosis and most appropriate management. This is exemplified in its most developed form by the recent WHO classification of tumours of the central nervous system, which for the first time, makes particular molecular features mandatory for rendering particular histopathological diagnoses.

In contrast, while cancers of the gastrointestinal tract are still diagnosed on the basis of their morphology, a few key molecular markers have been validated in classifying individual tumour types into molecular subgroups on the basis of their behaviour and responses to therapies. The fact that cancers of the gastrointestinal tract are so common renders these few key markers absolutely central in the management of human cancers.

Before any discussion of the specific details of the molecular pathology of any particular tumour, however, it is germane to review a handful of the challenges and caveats which are inherent to the practice of molecular pathology.

Above all else, it must always be borne in mind that in real-life practice, the vast majority of specimens received are small, must be reported within very short periods of time to benefit patients, and must be tested in the context of the robust quality controls to ensure good practice. This requires that molecular pathology practitioners are judicious in their use of tissue not only for molecular but also for immunohistochemical testing, and that well-organised logistics are in place.

Secondly, tumours are by definition genetically unstable, and contain enormous numbers of molecular alterations. In routine practice, however, only a limited number have been validated as having clinical significance. The difficulty, then, is to avoid being overwhelmed by the detail of the many thousands of recognised alterations, at the expense of the few which are known to be clinically valuable. In this vein, although the examination of large panels of genes or even sequencing of whole genomes certainly has a vital role in research and in recruitment into clinical trials, the assessment of extremely large banks of molecular alterations is currently of rather limited use in routine practice.

Thirdly, while molecular pathology certainly involves the interrogation of DNA sequences themselves, this is by no means the limit of its scope: abnormalities of chromosomal structure, of epigenetics and of protein expression have all been validated as predictive and prognostic markers in various tumours. It is incumbent upon molecular pathology services, therefore, to make use of multiple platforms for the full molecular profiling of tumours.

Furthermore, as the range of validated molecular alterations of all types in a given tumour increases — and especially given that many of them have complex interrelationships — their interpretation becomes exponentially more difficult, and the answer to the clinical question, 'what is best for this patient?', rapidly becomes unclear. This is made yet more complex by the fact that it is not molecular alterations alone which guide clinical interpretation: molecular alterations must be interpreted in the context of the tumour type and of the clinical scenario. The *BRAF* mutation (discussed in more detail later) perhaps exemplifies this best: the same mutation is predictive of response to targeted therapy in melanoma, but not in other tumour types (at least as a monotherapy, in colorectal cancers); it is, in contrast, a validated prognostic marker in microsatellite-stable (MSS) colorectal cancers and can be used as a diagnostic marker in equivocal thyroid lesions. This has an important bearing on the rationale for wider sequencing; algorithms for interpretation of any findings must be tumour-specific.

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At this point in the development of molecular pathology, therefore, there is a pressing need to perform assessment of molecular alterations in an algorithmic fashion, taking account of all molecular, morphological and immunophenotypic characteristics of a tumour, and the clinical circumstances of the patient. Even with only a few parameters, this form of algorithmic interpretation is quickly beyond the ability of an unaided human, and so the essential role of computation in cancer management is obvious.

In this review, we describe the clinically relevant molecular alterations of tumours of the gastrointestinal tract. By necessity — because different alterations are of different significance in different tissues — each organ of the gastrointestinal tract is examined in turn.

Oesophageal carcinomas

At present, in routine practice, there are no molecular alterations of proven clinical use in either oesophageal squamous cell carcinomas or adenocarcinomas.[1] Nonetheless, in both, there is early evidence that certain molecular markers may be of use in clinical management in the future.

For example, though controversial at present, there is evidence that human papillomavirus (HPV) is implicated in a subset of oesophageal squamous cell carcinomas, and this may — in future — be of interest in the context of immunotherapy.

After the fashion of gastric adenocarcinomas, the presence of *HER2* amplification in oesophageal adenocarcinomas arising in the context of Barrett's oesophagus predicts favourable response to the anti-*HER2* agent, trastuzumab (Herceptin®).[2] The details of this are as per gastric tumours, as discussed below.

Gastric adenocarcinoma

HER2 amplification

Trastuzumab has been licensed for a number of years for use in advanced gastro-oesophageal adenocarcinomas in combination with chemotherapy, following the promising the results of the ToGA trial,[2] in which *HER2* amplification was found in 22.1% of almost four thousand tumours. Prescription of this drug requires assessment for *HER2* amplification; the method employed in the ToGA trial was the classical two-step process with initial immunohistochemistry and confirmatory FISH for equivocal cases denoted 2+, as per the model of breast cancers. However, the scoring system used in gastro-oesophageal cancers is specific to tumours of this region — the Rüschoff—Hofmann system[3]; this differs from the scoring system in breast by taking account of the marked heterogeneity of *HER2* expression in these tumours, and has different criteria for assessment of biopsy and surgical resection specimens, as described in Table 1 and illustrated in Figure 1.

Two commercialised, validated assays are currently available: HercepTest™ from Agilent and VENTANA anti-*Her2-neu* (4B5) antibody from Roche. However, alternative methods such as in situ hybridisation techniques can be validated in-house and serve the same purpose. Additionally, technologies based on PCR (polymerase chain reaction) examining the DNA sequence itself represent possible alternatives.

In contrast to its predictive value, the prognostic value of *HER2* in gastro-oesophageal adenocarcinoma remains controversial, and currently the recommendation of the American Society of Clinical Oncology is that HER2 ought not to be used as a prognostic marker in such cancers.[4]

Molecular classification of gastric adenocarcinoma

Beyond *HER2*, there is no established molecular marker which has found use in the routine management of gastric adenocarcinomas. However, recent reviews — and particularly that from the Cancer Genome Atlas — have suggested a new classification of these tumours on the grounds of a small number of molecular alterations, in combination with their clinical and morphological features.[5] In this way, gastric adenocarcinomas have been divided into EBV-related, microsatellite instability-high (MSI-high), genomically-stable and chromosomally-unstable subtypes.

1. It has been known for many years that approximately 10% of gastric carcinomas are EBV-associated, especially proximal cancers, and there is evidence that this is an indicator of

The Rüschoff—Hofmann system for immunohistochemical assessment of *HER2* status in gastro-oesophageal adenocarcinomas. The means of assessment varies based on whether the specimen is a surgical resection or biopsy

Score	Staining pattern		*HER2* overexpression assessment
	Resection specimen	Biopsy specimen	
0	No reactivity or membranous reactivity in <10% of tumour cells	No reactivity or no membranous reactivity in any (or <5 clustered) tumour cell	Negative
1+	Faint/barely perceptible membranous reactivity in ≥10% of tumour cells; cells are reactive only in part of their membrane	Tumour cell cluster (≥5 cells) with a faint/barely perceptible membranous reactivity irrespective of percentage of tumour cells stained	Negative
2+	Weak to moderate complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster (≥5 cells) with a weak to moderate complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Equivocal (for FISH)
3+	Strong complete, basolateral or lateral membranous reactivity in ≥10% of tumour cells	Tumour cell cluster (≥5 cells) with a strong complete, basolateral or lateral membranous reactivity irrespective of percentage of tumour cells stained	Positive

Table 1

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