



Update on Colorectal Carcinoma

Dr Tony Watt

This article is designed to give a brief update of the pathogenesis of colorectal carcinoma and the rationale behind immunohistochemical stains for the DNA mismatch repair enzymes MLH1, MSH2, MSH6 and PMS2.

Colorectal carcinoma (CRC) is extremely common in Australia, representing approximately 14% of all cancers. It is second only to lung cancer as the most common cause of cancer death overall. Approximately 1 in 21 Australians will develop colorectal cancer during their lifetime, the risk increasing with age and rising sharply from the age of 50.

Identification of the adenoma-carcinoma sequence with its corresponding molecular genetic alterations, significantly increased our understanding of the pathogenesis of colorectal carcinoma. This route of carcinogenesis, hallmarked by genetic instability at the chromosomal level, is called the "suppressor pathway" because it is frequently accompanied by biallelic loss or inactivation of specific tumour suppressor genes (APC, p53 etc.). This sequence is typified by the inherited condition Familial Adenomatous Polyposis (FAP). In FAP there is a baseline (hereditary or germline) mutation in the APC gene and inactivation of the second (or normal-type) APC allele is thought to initiate the development of adenomas.

Not all genetic alterations involved in carcinogenesis in the colon however, fit into this linear tumour progression model. Some hereditary and sporadic CRC develop from a genetic instability at the DNA level. Non-encoding regions of the genome contain tracts of DNA with repetition of nucleotides that are especially prone to mismatch errors during DNA replication. DNA replication errors are normally repaired by a system of DNA mismatch repair proteins and DNA repair will not occur if the repair mechanism is disrupted through loss of these proteins. Loss of this DNA mismatch repair function leads to DNA replication errors (also described as microsatellite instability or MSI) and predisposition to not only colorectal ►

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Dr Watt graduated from the University of Queensland with honours in 1992. As a rural scholarship holder he then worked in provincial centres around Queensland. After spending a year in laboratory haematology at the Princess Alexandra Hospital in 1998, he then undertook specialist training in Anatomical Pathology. The initial years were spent at the Princess Alexandra Hospital before moving to QML Pathology as a senior registrar to complete his training.

Dr Watt has a special interest in gastrointestinal pathology, and has recently travelled to the USA and the UK to further this interest.

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► carcinoma but tumours in other organs (including the endometrium, small intestine, ureter and renal pelvis). This route of carcinogenesis in the colon is also termed the “mutator pathway”. Although it can be found in a subset of sporadic colorectal carcinomas, it is characteristic of Hereditary Nonpolyposis Colorectal Cancer (HNPCC or Lynch syndrome).

HNPCC is a precancerous autosomal dominant disorder that accounts for approximately 2 to 5% of colorectal carcinomas and is at least as common as FAP. It was initially defined by clinical and genealogical criteria (the Amsterdam criteria), but is now known to be caused by mutations in the DNA mismatch repair genes MLH1, MSH2, MSH6 and PMS2. Heterozygosity for a mutation results in susceptibility to cancer as loss of DNA repair proficiency follows inactivation of the second (or wild-type) allele. This leads to development of numerous DNA replication errors (microsatellite instability) and oncogenesis results from the mutational inactivation of additional genes implicated in the regulation of growth and differentiation. Patients with HNPCC have slightly larger numbers of adenomas than the remainder of the population and the adenomas are more often villous with areas of high-grade dysplasia. They are considered more likely to undergo malignant transformation.

Cancer with high levels of microsatellite instability (MSI-H) is the hallmark of HNPCC, but also occurs in approximately 15% of sporadic colorectal carcinomas. As opposed to HNPCC cancers, sporadic MSI-H carcinomas are thought to arise via methylation (and subsequent inactivation) of the promoter sequence of the DNA mismatch repair gene MLH1 (rather than mutation of the gene itself).

It is clearly important to be able to identify which people with colorectal carcinoma have HNPCC. Even among persons attending clinics for those at high risk for colorectal carcinoma, the sensitivity of the clinical (Amsterdam) criteria

Figure 1: Routinely sustained MSI-H cancer showing a pushing margin, mucinous differentiation and a surrounding Crohn's-like lymphoid infiltrate.

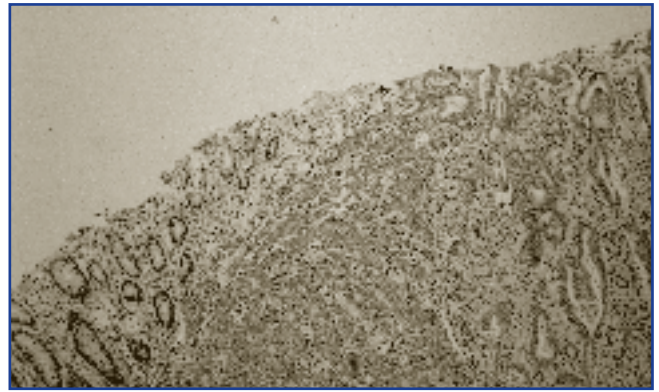


Figure 2: MSH2 immunohistochemical stain from a MSI-H cancer in a patient with HNPCC showing loss of staining in tumour nuclei. The adjacent normal mucosa and intervening lymphocytes show normal nuclear staining.

is only 40-80% and among unselected patients with colorectal cancer, the sensitivity is 50% or less. MSI-H cancers tend to have certain morphological characteristics (more often in the colon proximal to the splenic flexure; poor differentiation; mucinous differentiation; a pushing margin; intraepithelial lymphocytosis; peritumoural lymphoid and Crohn's like lymphoid responses). The morphological characteristics, however, have low sensitivity for identifying MSI-H cancers.

It is now possible to identify with a relatively high degree of sensitivity which colorectal carcinomas show microsatellite instability. Immunohistochemical stains can be performed on routinely processed colorectal cancers for DNA mismatch repair proteins MLH1, MSH2, MSH6 and PMS2. These proteins are present in adjacent normal colonic mucosa, but are selectively lost within tumour cells showing high levels of microsatellite instability. This is important for two reasons. Firstly it helps identify which tumours are likely to be associated with HNPCC. Secondly, identification of MSI-H carcinomas is important prognostically as they have been shown to be associated with (relative to microsatellite stable tumours):

1. An improved prognosis (stage for stage)
2. A 5-fold increased risk of developing a second colorectal carcinoma.
3. Potential resistance to some forms of adjuvant therapy (recent reports suggest MSI-H carcinomas do not benefit from adjuvant chemotherapy with fluorouracil).

For the above reasons immunohistochemical staining for MLH1, MSH2 and MSH6 is being routinely performed on colorectal carcinomas at QML Pathology and staining for PMS2 will be commenced in the near future.

For further information please contact Dr Tony Watt on (07) 3840 4644.

Soluble Mesothelin-Related Protein (MESOMARK®)

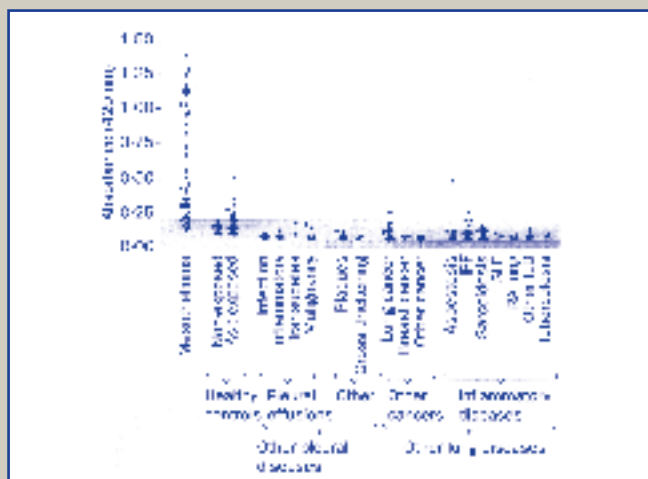
A Potential New Tumour Marker for Mesothelioma

The test for soluble mesothelin-related protein (SMRP) is a new test hailed as useful for the detection of mesothelioma, a highly aggressive tumour of serosal cavities.

Like all other “tumour markers”, it is not always positive in the presence of known mesothelioma (false negative) nor does a positive test absolutely indicate mesothelioma (false positive). However initial studies (Robinson et al., 2003) on a relatively small number of patients appear promising. The results are tabulated below:

Known mesothelioma	37 of 44 were positive
Known asbestosis without mesothelioma	1 of 22 were positive
Healthy controls: with known asbestos exposure	7 of 40 were positive*
with NO known exposure	0 of 28 were positive
All others	2 of 138 were positive#

* of these 7, 3 subsequently developed mesothelioma within 5 years
this last group includes 1 of 22 with bronchogenic carcinoma and 1 of 22 with fibrotic lung disease.



How can we use this test?

Much work is yet to be done, but by extrapolating from our experience with older established tumour markers, we can suggest:

1. In patients known to have mesothelioma and undergoing treatment, the blood level may be used to assess efficacy of treatment.
2. Although high-level occupational exposure to asbestos has essentially ceased, Australia still has many thousands of former asbestos workers with asbestosis of varying degrees of severity. This group is at risk of developing mesothelioma superimposed on their known disease. At present, screening for mesothelioma is costly or invasive. This blood test may assist in detecting a malignant change early when treatment may be more effective.

It would essentially select for patients in whom more expensive radiological or invasive cytological tests are indicated.

3. The biggest group of “at risk” people are the workers and property owners who have been exposed to asbestos through renovations, demolition etc. of older buildings.

Experience indicates that there is a negligible risk in living or working in these buildings. Installed, undisturbed asbestos-containing products are safe because the asbestos fibres are bound together in a solid matrix. But when they are cut, drilled, sanded or otherwise abraded with high-speed power tools, they can release asbestos fibres into the air. Prolonged exposure for months or years appears to carry the greater risk. For home owner/renovators, a brief one-off exposure poses a very low risk.

4. The largest group is the unexposed public. If the test was to be used at all in this group, it should be primarily confined to those who have declared themselves to have an above-average risk by presenting with suspicious clinical or radiological features.

I would express reservations about performing and interpreting the test in children. Their duration of exposure cannot reach the decades required for mesothelioma to develop, but more importantly, there has been no work to date about “normal levels” in the growing child. As many “tumour markers” simply reflect the change in formerly normal tissues toward a more immature or less well-differentiated form, tissue regeneration or growth is frequently associated with degrees of elevation even in the absence of malignant change. It would not be surprising if the “normal” levels in children were somewhat higher than those in adults.

In conclusion, we now have a potentially useful tool for the investigation of a particularly insidious and aggressive malignancy which has risen to prominence in recent times. However, like all new diagnostic and therapeutic modalities, it should be used with consideration while our experience grows.

Note: There is no Medicare item number for this test. As the reagent cost at this stage is particularly high, we are obliged to levy a non-refundable charge. With increasing use of the test, it would be hoped that the cost per test will fall.

For a detailed overview of the SMRP test please contact your local Medical Liaison Officer.

Goodpasture's Disease

Changes to Detection Methodology

Goodpasture's disease is an uncommon and serious autoimmune disorder characterised by pulmonary haemorrhage and crescentic glomerulonephritis. The onset is usually rapid with high fatality rates if untreated and persistent morbidity and sometimes mortality despite treatment. Diagnosis must be rapid to permit early intervention. Treatment usually involves plasmapheresis, and immunosuppression with high dose corticosteroids and cytotoxic therapy.

Anti-glomerular basement membrane antibodies (anti-GBM antibodies) with specificity for type IV collagen are present in as many as 95% of patients with Goodpasture's disease. Detection of these antibodies from serum provides a rapid diagnostic method for Goodpasture's disease, although definitive diagnosis is usually dependent upon histological examination of a biopsy (usually kidney and less commonly lung), including direct immunofluorescence examination of the biopsy for anti-GBM antibodies bound to the collagen containing basement membrane.

Investigations for suspected glomerulonephritis:

- Urinalysis
- Microurine
- Urine protein
- FBE
- CRP
- ELFT
- ANA
- C3 and C4 complement
- ANCA
- Anti-GBM

Consider if indicated:

- Cryoglobulins
- Hepatitis B and C serology if at risk
- Blood cultures
- Serum protein electrophoresis

QML Pathology is changing its methodology for the detection of anti-GBM antibodies. We have evaluated indirect immunofluorescence slide based testing, ELISA methodologies, and a newer detection method utilising chemiluminescent signal amplification (Pharmacia UniCAP). The UniCAP method demonstrates superior precision, accuracy,

Ordering of HDL/LDL Cholesterol

We are periodically reminded by the Health Insurance Commission that it is illegal for the laboratory to perform HDL/LDL analysis on a request for "LIPIDS".

To comply with the Schedule, HDL must be specified on the request form (and we report both HDL and LDL on a request for HDL). LIPIDS, LIPID STUDIES, FATS etc include only cholesterol and triglycerides.

We thank you for your understanding in this.

Dr David Drummond
Medical Director

sensitivity and specificity compared to the other assays. In addition, this method permits faster turn-around time and urgent processing of samples if required.

Since the 24 May 2005, anti-GBM antibodies have been measured by the UniCAP method. This assay provides quantitative results. The reference range or cut-off for normal is different to the current ELISA based assay. The new values are:

- Normal < 7
- Borderline 7-10
- Positive >10

If serial results are required, samples positive on the old assay can be rerun in parallel with current samples on the new assay.

For further information about anti-GBM antibodies, please contact Dr David Heyworth-Smith on (07) 3840 4444.



Opening Announcement for Toowoomba Laboratory

After much anticipation, on the 16th May QML Pathology opened its newest laboratory in Scott Street, Toowoomba. The availability of localised testing services will allow QML Pathology to offer a significant service to Toowoomba practitioners and their patients. Adjoining the new laboratory is an additional collection centre which will provide another accessible option for patients in Toowoomba.



The new Toowoomba Laboratory
Scott St, Toowoomba

This further commitment brings QML Pathology's local network to 3 collection centres and 1 laboratory in Toowoomba, complementing a second laboratory in Stanthorpe and 3 clinics in the surrounding region. This addition continues the growth seen throughout our other centres in regional Queensland and northern New South Wales. The opening of the laboratory ends months of preparation, hard work and expectation, delivering a facility that meets the needs of the Toowoomba medical community. Our couriers will continue to collect specimens as previously, however most specimens will be assessed and reported locally providing significant increases in efficiency.

The new lab will be able to perform a broad range of pathology testing capable of streamlining the service available in the region, lowering turn around times on the majority of tests. The laboratory will be operational Monday to Friday 8.30am - 4.30pm and on call 24 hours a day, 7 days a week, further reducing delays in result reporting.



Testing being performed in the new
Toowoomba Laboratory

The new laboratory will be serviced by Dr Dean Townsend. Dr Townsend has been with QML Pathology since 1997 and is the pathologist in charge of our Ipswich and Stanthorpe laboratories, now adding Toowoomba to his portfolio. Dr Townsend's colleagues at the Toowoomba laboratory also possess strong experience in the operation of an efficient pathology service.

The opening of the Toowoomba lab has been an exciting step forward in the area for QML Pathology and one which we have looked forward to for some time. Scott Kane, Medical Liaison Officer for Toowoomba, will be seeing many of you in the near future and is available to assist with any queries, concerns or service requirements on 0413 271 231.



Dr Dean Townsend,
Pathologist in Charge



Scott Kane,
Medical Liaison Officer

COLLECTION CENTRE NEWS

For the convenience of our doctors and patients, we have listed the latest additions and changes to QML Pathology's network of clinics:

NEW CLINICS

Robina (07) 5593 3682

Shop A 481, Robina Town Centre,
Robina Town Centre Drive
Mon - Fri 7.30am - 1.00pm
2.00pm - 4.00pm

Toowoomba (Scott St) (07) 4638 9149

Cnr Scott & Curzon Sts
Mon - Fri 8.30am - 4.30pm

CLINIC CHANGES

Beaudesert (07) 5541 4729

Lavender House, 37 William Streets
Mon - Fri 7.30am - 12.30pm

Clifton Beach (07) 4055 3013

Clifton Village Shopping Centre
Captain Cook Highway
Mon - Fri 8.00am - 12noon

Cooroy (07) 5447 6949

Cooroy Specialist Medical Centre
50 Maple Street
Mon - Fri 7.00am - 12.30pm
1.00pm - 4.30pm

Fortitude Valley (07) 3257 4187

Central Brunswick Shopping Village
421 Brunswick Street
Mon - Fri 8.00am - 1.00pm

North Mackay (Greenfields) (07) 4943 7833

Shop 4A, Greenfields Mt Pleasant
Shopping Centre, Grandview Dr
Mon - Fri 8.00am - 1.00pm
2.00pm - 4.30pm

Kallangur (07) 3204 6711

1440 Anzac Avenue
Mon - Fri 7.00am - 5.00pm
Sat 8.00am - 11.30am

McDowall (07) 3353 6069

McDowall Village
Cnr Beckett & Hamilton Roads
Mon - Fri 8.00am - 12noon

Sandgate (07) 3269 2693

1st Floor, Bon Accord Shopping Centre
12 Lagoon Street
Mon - Fri 7.00am - 3.00pm

Taringa (07) 3371 3266

First Floor, 15 Morrow Street
Mon - Fri 7.00am - 12noon
12.30pm - 7.00pm
Sat 8.00am - 2.00pm
Sun 8.00am - 12noon

Tugun (07) 5598 9160

Ground Floor, John Flynn Medical Centre
Inland Drive
Mon - Fri 7.30am - 5.00pm

Aptima Assay Update New Testing Method for CT and NG

Recently you have received information regarding the introduction of the Aptima Combo 2 (AC2) assay, a new testing method for the molecular diagnosis of Chlamydia trachomatis and Neisseria gonorrhoeae from male and female urines and genital specimens. Specimen Collection guides and stock has begun to filter into medical practices for this new testing method. With the arrival of stock we would like to confirm that the Aptima Swab packs contain 1 blue swab (in packet with green writing) for both male and female specimens, 1 white swab (in packet with red writing) for cleaning excess mucus and 1 transport tube. The swab specimens (blue - for male and female) should be inserted into the accompanying tube of liquid preservative immediately after collection. The dry swabs currently used are not considered optimal for the new assay. The collection tube containing the liquid preservative is specific for this assay, and is unsuitable for all other molecular tests.

If you require further information please contact you local Medical Liaison Officer.

Doctors' Notice Board

Dr. David Heyworth-Smith (Clinical Immunology and Allergy) has a new phone number (07) 3847 3256. The practice address is unchanged within The Greenslopes Private Hospital.

Dr Paul Bartley would like to inform colleagues that he has moved his base from Stoneham Chambers at Stones Corner to:

Ground Floor Specialty Clinics
Greenslopes Private Hospital
Newdgate Street, Greenslopes 4120
Ph: (07) 3847 3256 (All appointments & enquiries)
Fax: (07) 3847 3257

He will also consult on a sessional basis at the Wesley Medical Centre.

Most of his clinical work will remain inpatient-based and he visits the Wesley, Greenslopes, Mater Private, St Andrews & Brisbane Private.

This newsletter has been prepared and published by QML Pathology for the information of referring doctors. Although every effort has been made to ensure that the newsletter is free from error or omission, readers are advised that the newsletter is not a substitute for detailed professional advice.

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