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AMPATHCHAT

Dr Robert M. Maritz, FC Path (SA) Anatomy
Dr Jaysen Knezovich, PhD Genetics, Principal Medical Scientist

Molecular testing in colorectal carcinoma

Introduction

Colorectal carcinoma (CRC) is the third-most commonly diagnosed potentially fatal cancer worldwide and is the third leading cause of cancer-related death.[1,2] Despite advances in the chemotherapeutic and surgical treatment of CRC, the disease continues to have a relatively poor prognosis, with a five-year survival rate of 55%.[3] Recent advances in the molecular testing of CRC have provided new insights into the complexities of this disease at a genetic and epigenetic level. Furthermore, with the increased use of personalised adjuvant chemotherapy, molecular testing has become increasingly important, as the results of such tests often dictate the choice of therapy.

This newsletter will explore the use of selected molecular tests in CRC and discuss the clinical and prognostic implications of these tests.

Molecular pathways involved in colorectal carcinogenesis

Colorectal carcinomas arise from colorectal epithelium, which has undergone a series of molecular changes, referred to as the adenoma-carcinoma progression sequence.[4] With increasing genetic mutations, progression from normal epithelium to adenomas with dysplasia occurs. Eventually, adenomas that show high-grade dysplasia give rise to invasive colorectal carcinomas.

Three main molecular pathways are involved in colorectal carcinogenesis.[5]

- The first is the chromosomal instability pathway. Most sporadic colorectal carcinomas arise along this pathway, as well as syndromic CRCs other than those due to Lynch syndrome (hereditary non-polyposis colon cancer). The most commonly mutated gene in this pathway is APC, with more than 90% of tumours arising along this pathway. This demonstrates an APC mutation. Other mutated genes include KRAS, TP53 and PIK3CA.

- The second pathway is the microsatellite instability (MSI) pathway. Approximately 15% of sporadic CRCs arise along this pathway, together with tumours occurring in the context of Lynch syndrome.[4] Microsatellites are small, repetitive DNA sequences that are found throughout the genome.[6] They are particularly prone to errors during DNA replication. These errors are identified and corrected by mismatch repair genes. Should these genes be defective or deficient, a situation known as *microsatellite instability* occurs, whereby the length of a microsatellite sequence is altered. If the microsatellites occur in genes that affect cell growth, carcinogenesis can result.

- The third pathway is the so-called CpG island methylator phenotype (CIMP).[5] CpG islands are regions of genes rich in the dinucleotides cytosine and guanine. Methylation of these regions can lead to the silencing of tumour suppressor genes, as well as genes involved in mismatch repair.

Other genes commonly mutated in these tumours include KRAS and BRAF.

KRAS

KRAS is an oncogene. When mutated, it confers a growth and survival advantage on the cell.[4] Mutations in KRAS occur as an early molecular event in colorectal carcinogenesis. KRAS forms part of the epidermal growth factor (EGF) signalling cascade (see Figure 1), and is mutated in both the chromosomal instability and the CIMP pathways.

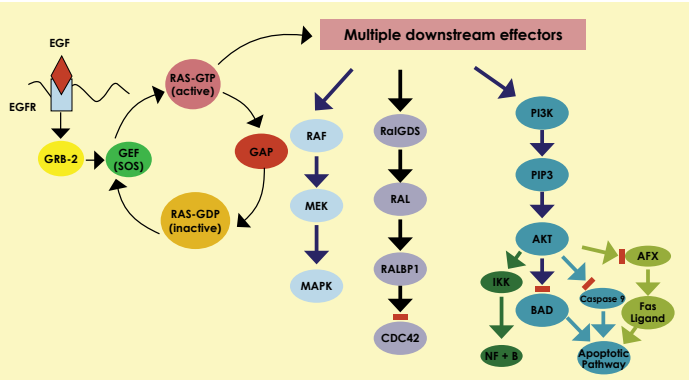

Figure 1: The EGF, RAS and RAF signalling pathways.[4]

Recently, monoclonal antibodies directed against the EGF receptor (EGFR) have been developed, and have proven to be beneficial in the treatment of patients with advanced CRC. These drugs include cetuximab, bevacizumab and panitumumab.[3] At the time of writing, only cetuximab was available in South Africa.

Testing for the presence of specific point mutations in the KRAS gene is of vital importance in CRC, as tumours that harbour these mutations are resistant to the abovementioned therapies. This is because KRAS acts downstream of EGF. Blocking EGFR will therefore have no effect in the presence of continued KRAS activation.

Initially, only mutations in codons 12 and 13 of the KRAS gene were thought to be responsible for anti-EGFR resistance. Subsequent studies, however, have shown that mutations in KRAS codons 59, 61, 117 and 146, and NRAS codons 12, 13, 59 and 61 also indicate resistance. Testing for mutations in all the aforementioned codons is now recommended prior to the commencement of anti-EGFR therapy in patients with advanced CRC.[3]

Besides guiding the choice of chemotherapy, KRAS mutation analysis provides prognostic information, in that tumours that harbour these mutations have a worse prognosis, with reduced disease-free and overall survival.[7] As KRAS mutations usually occur early in the carcinoma sequence, testing can be performed on both primary and metastatic tissue.

BRAF

BRAF is a serine or threonine kinase that acts downstream of KRAS (see Figure 1). The most common BRAF mutation is a valine to glutamine substitution at codon 600, known as the V600E mutation.[8] BRAF and KRAS mutations are mutually exclusive in CRC.

Besides CRC, BRAF mutations have been identified in melanomas, thyroid carcinomas, non-small cell lung carcinomas, cholangiocarcinomas and certain gliomas. Specific BRAF inhibitors have been developed, including vemurafenib, dabrafenib and encorafenib. These therapies have been shown to be extremely useful in patients with the abovementioned tumours.

In CRC, however, BRAF inhibitors have a response rate of less than 5%.[8] This is thought to be due to the feedback activation of EGFR. Furthermore, tumours with BRAF mutations show little response to anti-EGFR therapy. The chemotherapeutic options for patients with these tumours are thus limited. Studies combining BRAF-inhibitors and EGFR-inhibitors are ongoing.[8]

BRAF-mutated CRCs are unique in that they present with frequent peritoneal metastases. These tumours have a significantly shorter overall survival than BRAF-wild-type tumours.[8]

Microsatellite instability

The major mismatch repair genes include MLH1, MSH2, MSH6 and PMS2. Loss of function of any of these genes leads to microsatellite instability (MSI), as discussed briefly above. Alteration in these genes can be sporadic or inherited.[6]

Up to 15% of sporadic CRCs shows MSI. This is due to promoter hypermethylation of the MLH1 gene, which leads to gene silencing and loss of function. Many of these tumours have the so-called CIMP phenotype.

Lynch syndrome is an autosomal dominant condition that occurs in patients with a germline mutation in any one of the mismatch repair genes (usually MLH1 or MSH2).[9] Besides the risk of CRC, these patients are prone to developing tumours of the endometrium, stomach, liver and brain, among others.[6]

CRCs with microsatellite instability usually occur in the right side of the colon, and have unique histological features, including a medullary or mucinous morphology, pushing border and a peritumoral Crohn-like response.[6] Despite many of these tumours having a superimposed BRAF mutation, they have been found to have a better prognosis than MSI-stable tumours.[9] Given the relatively good prognosis of these tumours, some authors advocate observation rather than postoperative chemotherapy in patients with stage II MSI tumours.[7]

Recently, an anti-Programmed Death 1 (PD-1) monoclonal antibody, pembrolizumab, was developed.[11] The PD-1 pathway is a system that inhibits the Th1 cytotoxic immune response. Inhibition of this pathway has been shown to be beneficial in patients with advanced cancers such as CRC, non-small cell lung carcinoma and melanoma. In advanced CRC, the presence of microsatellite instability predicts increased responsiveness to pembrolizumab.

Screening for microsatellite instability can be performed using immunohistochemistry or polymerase chain reaction. Immunohistochemistry has several advantages, including being more cost effective and readily available. Furthermore, depending on the results, immunohistochemistry might be able to predict the mismatch repair gene involved. Should a patient be suspected of having Lynch syndrome, sequencing of the mismatch repair gene(s) will need to be performed in order to confirm the presence of the disease and allow for the testing of relatives.

Importantly, BRAF mutations are found exclusively in sporadic MSI tumours and not in Lynch syndrome.[4] The finding of a BRAF mutation in an MSI tumour therefore obviates the need for gene sequencing as part of an investigation for Lynch syndrome.

Future directions

Currently, mutational analysis in CRC is performed on selected tumours only. Given the decreasing costs associated with next-generation sequencing in the future, the universal testing of all CRCs may become feasible.[2,10]

The advantage of this strategy, especially if panels of tests are employed, is that multiple genes could be tested concurrently. This would not only guide therapy. It would ensure that no patient with an inherited CRC syndrome will go undiagnosed.

Conclusion

Mutational analysis in CRC is able to provide extremely useful information to a multidisciplinary team involved in patient care. Some mutations signify a better or worse prognosis. Some indicate an inherited syndrome that allows for the testing of family members. Some guide chemotherapeutic management. Figure 2 provides a simple yet useful testing algorithm that could be employed in the molecular workup of colorectal carcinomas.

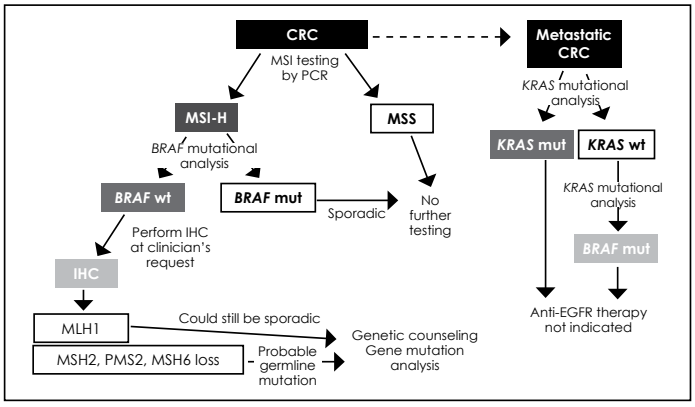

Figure 2: Molecular testing algorithm.[6]

Abbreviations: MSI: microsatellite instability; MSS: microsatellite stable; mut: mutated; wt: wild type.

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Genetic testing for colorectal cancer associated genes

Ampath Genetics offers a range of test options for screening genes associated with colorectal cancer. These include both prognostic, acquired somatic mutations and inherited (germline) mutations.

Somatic mutations

Screening is available for somatic (acquired) mutations in the following genes:

KRAS
BRAF
NRAS
PIK3CA
TP53

Inherited mutations

Screening is available for inherited (germline) mutations using a next-generation sequencing panel, targeting the following genes:

APC
BMPR1A
DPYD
EPCAM
GREM1
MLH1
MLH3
MSH2
MSH6
MUTYH
NTHL1
PMS1
PMS2
POLD1
POLE
SMAD4
TGFBR2

For any information on test options and approaches, please contact 012 678 1361 or ngs@ampath.co.za.

Interrogating the genome from the lowest to the highest resolution

Introduction

Ampath Genetics is based at the National Reference Laboratory (NRL) in Centurion, Gauteng, South Africa, and offers a full in-house complement of clinical and laboratory genetic services. The Ampath Genetics Laboratory is ISO 15189 accredited, participates in several external quality assessments on an ongoing basis, and is an HPCSA-approved training facility for medical scientists and technologists. Ampath Genetics is comprised of skilled and experienced HPCSA-registered medical scientists, medical technologists, clinical geneticists and genetic counsellors. The Ampath Genetics Laboratory offers an extensive range of genetic techniques, which allow interrogation of the genome from whole chromosomes down to single nucleotides of DNA. These techniques make use of advanced genetic technologies, including next-generation sequencing (NGS).

Service offering

Ampath Genetics offers tests that can be used for predictive, diagnostic and prognostic purposes, which include (but are not limited to) cancer genetics (breast, ovarian, leukaemia, lymphoma), reproductive genetics (invasive prenatal testing, non-invasive prenatal testing (NIPT), products of conception), drug metabolism, haematology, neurology, carrier screening and paternity testing.

Level I: Cytogenetics (chromosomal analysis)

Chromosomal analysis (karyotyping) represents one of the oldest investigative genetics approaches, which offers a bird's eye view of the genome at a low resolution. This hands-on technique requires significant expertise to analyse the chromosome complement of cultured cells in metaphase for large structural and/or numeric abnormalities.

Level II: Cytomolecular genetics (fluorescence in situ hybridisation/FISH)

FISH offers a higher resolution approach than conventional chromosomal analysis via karyotyping. FISH makes use of fluorescent probes, which bind to targeted regions of chromosomes, to determine the presence, absence and/or genomic location of these regions. Oncologists, haematologists and histopathologists are generally most knowledgeable about the various probe regions of interest for specific clinical situations in their respective fields. Metaphase FISH analysis on cultured cells can be applied to elucidate a variety of cytogenetic findings, including microdeletions and amplification events associated with specific cancers.

Level III: Cytogenomics (array comparative genome hybridisation/aCGH)

Array CGH is a molecular level investigation (not requiring light microscopic analysis as used for chromosomal analysis), which offers a genome-wide, high-resolution view of chromosomal structure. The genomic regions analysed are smaller than those detectable by FISH analysis, but are still significantly larger than the resolution that can be studied when using sequencing technologies. Specialised software and databases are required to interpret the clinical significance of observed genetic variants. aCGH testing currently represents a first-tier investigative approach for leukemias known to be associated with copy number variations (such as CLL), autism spectrum disorders, undifferentiated neurodevelopmental delay and clinically difficult dysmorphic syndromes and familial/syndromic epilepsies.

Level IV: Molecular genetics

Molecular genetic testing utilises DNA technologies, including PCR and sequencing, to perform gene-level analyses. Such analyses can attain resolution down to single nucleotides of DNA. The ability to identify specific mutations can be of significant clinical value. Applications of such analyses include identifying mutations for targeted drug therapies in relevant cancers, minimal residual disease determination in oncology, DNA profiling analyses for parentage/sibling investigations and forensic profiling. Molecular genetic techniques are also currently utilised for pregnancy loss investigations.

Level V: Next-generation sequencing (NGS)

NGS is high-throughput sequencing that is capable of rapidly generating vast amounts of genomic data. NGS can provide information on gene expression alterations, chromosome copy number aberrations, and single base changes in DNA. Test designs and data analyses have to be performed by means of special software programs, and a high level of skill is required to ascribe pathological significance to the findings. As a consequence, this testing modality demands increased input from clinical geneticists and genetic counsellors with regard to conveying the information to individuals or families and their doctors. With an NGS approach, any number of genes can be incorporated into custom-designed panels required by pathologists or clinicians, allowing a single assay determination of all clinically relevant genes for conditions that include abnormal iron storage, primary immune deficiencies and breast cancer.

Genetic counselling and clinical genetics

Genetic counselling is a holistic healthcare service that aims to assist healthcare professionals and patients in decision-making related to genetic testing. Genetic counselling offers insights into the options and availability of testing for genetic conditions, including costs and turnaround times, and aids in obtaining appropriate informed consent.

This usually includes a review of the therapeutic, management, pregnancy and lifestyle options available, following a final diagnosis. Appropriate management for many genetic testing scenarios requires both pre-test and post-result consultations with patients and/or their families. These consultations require a multidisciplinary approach, where the genetic counsellor acts as a facilitator of the various disciplines involved, so that the patient can be offered a cohesive medical management plan. Genetic counselling is offered at the request of, and in collaboration with, the patient's primary physician(s).

The clinical geneticist supports the genetic counsellor during some of the above processes, assists with suggesting or refining clinical diagnoses for a more focused and cost-effective laboratory testing approach, sometimes directs the investigative approach to be adopted in non-routine situations and, together with the genetic counsellor, is responsible for translating laboratory findings of assessed individuals or families into practical and actionable clinical report findings.

For more information

General queries: 012 678 1350
Clinical genetics: 012 678 0645
Genetic counselling: 012 678 1362