Genetic aspects of colorectal cancer and its impact on disease management

Aspectos genéticos do câncer colorretal e seu impacto no manejo da doença

Fernanda Cardoso Parreiras¹, Gustavo Martins Zola Santiago¹, Alexandre Martins da Costa¹, Antônio Lacerda Filho²

DOI: 10.5935/2238-3182.20130034

ABSTRACT

Several studies seek to enhance diagnosis and treatment of colorectal cancer (CRC) in order to reduce its incidence and mortality. New technologies proposed include the analysis of fecal DNA, therapies aimed at specific molecular targets and determination of therapeutic response and prognosis with genetic analysis. This study aims to review these new achievements and present them so that they can be used in a practical and objective way.

Key words: Colorectal Neoplasms/genetic; Colorectal Neoplasms/diagnosis; Colorectal Neoplasms/therapy; Genetic Testing.

RESUMO

Diversos estudos buscam agregar métodos de diagnóstico e de terapêutica com o intuito de diminuir a incidência e a morbimortalidade do câncer colorretal (CCR). Novas tecnologias propostas para esse fim decorrem da análise do DNA fecal, terapias voltadas para alvos moleculares específicos e determinação de resposta terapêutica e prognóstico com a análise genética. Este estudo procura revisar essas novas conquistas e apresentá-las para que possam ser usadas de forma prática e objetiva.

Palavras-chave: Neoplasias Colorretais/genética; Neoplasias Colorretais/diagnóstico; Neoplasias Colorretais/terapia; Testes Genéticos.

INTRODUCTION

Colorectal cancer (CRC) figures among the five most common causes of death by cancer, barring non-melanoma skin tumors.¹² Its sporadic form represents 70% of the cases of colorectal cancer and is mostly found in individuals over 50 years of age. Deoxyribonucleic acid (DNA) lesions are often caused by interaction with the environment or by the effects of aging. Hereditary CRC occurs in individuals who inherit an alteration in a tumor-suppressing gene. Hereditary predisposition for CRC is found in less than 10% of the patients.¹

GENETIC BASES FOR COLORECTAL CANCER

Cell mutations can occur spontaneously during its growth and development, and a somatic mutation can result in proliferation of cells with altered (mutated) genetic ma-
Genetic aspects of colorectal cancer and its impact on disease management

Most of the genes maintain their functions, even if one of the alleles is inactivated. For the gene to lose function and the proliferative process to start, characteristics of a neoplasm, both alleles must be inactivated.

In the case of sporadic CRC, both alleles must be inactivated by fortuitous genetic events, such as somatic mutations, deletions or hypermethylation. In general, several decades are necessary for both alleles of the genes involved in the mechanism of tumor genesis to be lost, which may explain the higher frequency of sporadic CRC among older individuals.

Mutated genes in the different human tumors belong to three different classes: oncogenes, tumor-suppressing genes and DNA repair genes (MMR-DNA Mismatch Repair genes).

GENES INVOLVED IN COLORECTAL CANCER

Oncogenes

Protooncogenes are genes that produce the proteins that promote cellular growth and proliferation. The mutations found in them cause gain of function and may be caused by the mutation in a single allele.

The main oncogenes involved in CRC are genes from the RAS family, composed by three genes – K-RAS (v-Ki-ras2 Kirsten rat sarcoma 2 viral oncogene homolog), H-RAS (v-Ha-ras Harvey rat sarcoma viral oncogene homolog) and N-RAS (neuroblastoma RAS viral (v-ras) oncogene homolog), which codify small proteins (21 kDa) that connect to guanine triphosphate (GTP). RAS proteins, also called p21, are located in the cellular membrane.

Tumor-suppressive genes

Tumor-suppressive genes produce proteins that inhibit tumor formation by regulating mitotic activity, which has an inhibitory control over the cellular cycle. Tumors can proliferate when those inhibitory controls are deregulated by mutation. The main tumor-suppressive genes related to the colorectal cancer are the APC and p53 genes.

The initial mutations in the sequence adenoma-carcinoma occur in the APC gene. The earliest phenotype alteration is known as "aberrant crypt formation" and these cells' typical genetic aberration is the formation of abnormally short proteins, known as APC truncation or truncated proteins. Most of the clinically relevant disturbances related to APC are truncation mutations created by inadequate transition of premature termination codons.

Mutations in gene p53 (tumor protein p53) located on the short arm of chromosome 17 (17p13) are identified in 50% or more of colorectal tumors and are mostly found between exons 5 and 8 of that gene. That mutation engenders inactivation of gene p53 and that is why, sometimes, it coincides with a transition from adenoma into invasive carcinoma in CRCs. The identification of a mutation in gene p53 is important for the prognosis of CRC, since suggests worse prognosis and shorter survival for patients with tumors.

Deletions in chromosome 18's long arm (18q21) were found in 70% of the CRC cases, and this is the location for the "deleted in colorectal carcinoma" gene (DCC). It is believed that this gene encodes a protein that acts upon cell-cell and cell-matrix interactions, and its mutation is related to the occurrence of the so-called advanced adenomas.

Repair Genes

The DNA repair system operates during its replication process and is responsible for the high-fidelity substitution of nucleotides that may present incorrect parameters. Inactivation of that system can lead to erroneous accumulation of paired nucleotides and the predisposition to carcinogenesis.

Mutations in those genes are related with hereditary nonpolyposis CRC syndrome (HNPCC), also known as Lynch syndrome. Tumor formation requires inactivation of the repair system followed by other mutations, such as the mutation in APC gene. Mutations in the repair genes cause microsatellite instability (MSI), which are repetitive DNA sequences that seem to be randomly distributed along the genome. The stability of these sequences is a good measure of DNA integrity. The tumors that have such instability are classified as replication error tumors (RER+).

Modifying Genes

In the last decades, there has been an extraordinary increase in knowledge of the carcinogenic pathways involving cyclooxygenase and the pathogenesis of...
Genetic aspects of colorectal cancer and its impact on disease management

CRC. Chronic use of acetylsalicylic acid (ASA) reduces the incidence of adenomas and CRC. The main target of nonsteroidal anti-inflammatory drugs (NSAIDs), ASA included, is the cyclooxygenase enzyme (COX), involved in the production of prostaglandins and other eicosanoids from arachidonic acid. Two isoforms of the COX protein are identified: COX-1, which is a constituent of normal cells; and COX-2, an induced gene associated with inflammation and carcinogenesis.11

The COX-2 gene represents an expression increase of approximately 43% for adenomas and 86% for carcinomas. COX-2 expression probably influences angiogenesis and apoptosis regulation.1 The use of sulindac, a non-specific COX inhibitor, promotes polyp regression in patients with familial adenomatous polyposis.12 While this effect is also observed with prescriptions of COX-2 inhibitors, use of these drugs is discouraged due to potential cardiotoxicity.

Preliminary studies show that the gene peroxisome proliferative activated receptor (PPAR) may be involved in signal transduction mediated by gene APC and with COX pathway.10

Table 1 shows a summary of the genes involved in CRC carcinogenesis, their frequency and main actions.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Frequency</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td>85%</td>
<td>Associated with Familial Hereditary Polyposis; inactivation found in 85% of colorectal sporadic cancers.</td>
</tr>
<tr>
<td>MLH1, MLH2, MLH6</td>
<td>15-25%</td>
<td>Associated with hereditary colorectal cancers in HNPCC polyposis.</td>
</tr>
<tr>
<td>TP53</td>
<td>35-55%</td>
<td>Associated with transition from polyps to invasive cancer as last key to the process</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>25-30%</td>
<td>Mutation present in 80% of tumors with microsatellite instability and 15% of colorectal cancers with microsatellite stability.</td>
</tr>
<tr>
<td>SMAD4</td>
<td>10-35%</td>
<td>Mutation found in Juvenile Familial Polyposis with increased risk of colorectal cancer in over 60% of the cases after the third or fourth decade of life.</td>
</tr>
<tr>
<td>KRAS</td>
<td>35-45%</td>
<td>Patients with stage IV colorectal and KRAS mutation cancer do not respond to therapy with EGFR inhibitors.</td>
</tr>
</tbody>
</table>

COLORECTAL CANCER GENETICS AND HEREDITARY SYNDROMES

Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is a dominant autosomal hereditary syndrome characterized by the presence of hundreds of thousands of colorectal cancers.
Genetic aspects of colorectal cancer and its impact on disease management

Familial juvenile polyposis

It is an autosomal dominant hereditary syndrome characterized by the presence of polyps formed by components of the stroma (hamartoma). Germline alterations have been identified in genes PTEN and SMAD4 genes in these patients. It was recently associated to a high risk of colorectal cancer.3,21

Lynch Syndrome or hereditary nonpolyposis CRC syndrome (HNPCC)

This is a syndrome related to germ line mutations in repair genes (mismatch), which include colorectal carcinoma, breast carcinoma, endometrial carcinoma, and ovarian carcinoma among other types of neoplasms. It is assumed that approximately 6-8% of CRC cases may be related to HNPCC.17

It is characterized, differently from FAP, by the occasional presence of a small number of polyps, often located on the right side. The progression of those polyps into carcinomas is fast, probably because they start with mutations in the DNA repair genes and are quickly transformed when mutations in gene APC are noted, in a phenomenon known as accelerated tumorigenesis.17

Besides diagnosis by genetic testing, the Lynch syndrome can be clinically diagnosed by Amsterdam criteria, originally introduced in 1990 (Table 2). However, because they were found to be too restrictive, the Bethesda criteria (Table 3) were proposed, and have been used as parameters for prescribing the microsatellite instability test, basis for the molecular diagnosis of this hereditary syndrome.1,3

Table 2 - Amsterdam Criteria

| At least three cases of colorectal cancer (CRC) that fulfill the following criteria: |
| A family member is in 1st degree relation with the other two; |
| At least 2 successive generations affected; |
| At least one of the CRC cases diagnosed under the age of 50; |
| Familial adenomatous polyposis should be excluded. |

Peutz-Jeghers Syndrome

It is an autosomal dominant syndrome characterized by mucocutaneous melanotic pigmentation associated with multiple polyps in the gastrointestinal tract. It is determined by germline mutation in the gene STK11/LKB1. It is a rare syndrome of barely any relevant clinical importance compared to HNPCC and PAF.20

Genetic diagnosis of colorectal cancer

Mutation detection methods can be divided into direct and indirect methods. Direct methods such as DNA sequencing reveal the existence of a genetic variant and its exact nature. Although DNA sequencing is the gold standard for analyzing several genes, it is still a very demanding and costly method, which is why indirect methods have been developed. These include denaturing high performance liquid chromatography (DHPLC) amplification by polymerase chain reaction (PCR). When compared to the cost of direct sequencing, the analysis of a fragment is about 10 times less costly, and eight times faster, with DHPLC.22

Truncated protein test (TPT) is another indirect method that can be used and is based on differences in migration of an altered protein in gel electrophoresis. That test is performed on a RNA extraction from the patient, often from leukocytes, and is the most
indicated in the diagnosis of PAF, with sensitivity of approximately 65%.

The genetics of HNPCC is more complex than that of PAF because germinative mutations in any of the genes involved in DNA repair can lead to the emergence of this syndrome. This way, microsatellite instability test should be the first choice for the diagnostic investigation of Lynch Syndrome, and it may be performed using PCR or, preferentially, by immunohistochemistry. A positive result, particularly indicating high frequency of instability (MSI-H), implies the need for direct determination of the mutation through DNA sequencing.

PROGNOSTIC MARKERS IN COLORECTAL CANCER

Already established relationships between a given genetic alteration and disease prognosis have been evaluated for potential as a test and some markers are already in use to that end. It is known that mutations in genes APC, hMLH1 and hMSH2 indicate that carriers have increased risk for developing CRC and prophylactic colectomy may be recommended. Moreover, deficient DNA repairing is, in general, associated with better prognosis and improved response to adjuvant chemotherapeutic treatments based on the use of 5-fluorouracil.

Finally, another use for this kind of technology is in determining k-RAS mutation in patients with stage IV CRC. Patients with this mutation do not respond to the inhibitory therapies of epidermal growth factor receptors (EGFR), such as cetuximab.

NEW GENE THERAPIES FOR COLORECTAL CARCINOMA

The exponential increase in the knowledge of tumor molecular biology and in the characterization of the genes involved in the genesis and progression of cancer provides very promising therapeutic options. Due to the intense research on the genetic base of CRC, there is growing optimism regarding the possible uses of knowledge about molecular biology in creating new and effective ways to fight this disease. Current gene therapy still falls short of replacing the numerous mutating genes related to CRC, but is already able to address some important drug therapy approaches.

Researches have been focused on developing therapies that target the product of genes with mutations. Therapy directed against the epidermal growth factor receptor (EGFR), purified and characterized by Cohen in 1980, has become a reality with the creation of anti-HER-1/EGFR antibodies such as cetuximab, as well as ABX-EGF, EMD72000, H-R3 and MDX-447 and anti-HER-2/neu, such as trastuzumab and 2C4. Agents that act directly on the tyrosine-kinase enzyme are also available, such as gefitinib and OSI-774. Because these are agents that specifically block in the critical ways the development and progression of neoplastic cells, a new perspective on cancer therapy is now at our reach.

Epidermal growth factor receptor (EGFR) is a glycoprotein of the plasma membrane composed of an extracellular binding domain, a lipophilic transmembrane segment and an intracellular tyrosine-kinase domain. EGFR is a member of the HER growth factors that include HER-1, HER-2, HER-3 and HER-4, important mediators for cell growth, differentiation, and survival. The activation of the complex EGFR-tirosine-kinase has been identified as an initiating event in the intracellular signaling cascade leading to proliferation, survival, angiogenesis, and metastasizing. Kari et al. demonstrated that malignant cells make inadequate contact with the stroma matrix, requiring activation from the EGFR so they can survive. Nevertheless, a lot of controversy surrounds the actual benefits of these new therapies, mainly regarding the use of drugs against epidermal growth factor receptors (EGFR) for the treatment of colorectal cancer.

Recently, German researchers have developed a randomized study to check whether the best global response rate of cetuximab in combination with oxaliplatin, leucovorin and fluorouracil (FOLFOX-4) was higher than that of FOLFOX-4 alone, as a first-line treatment against metastatic CRC. The influence of K-RAS mutation status was investigated by extracting tumor DNA and carrying out PCR. It was found that patients with wild variants of K-RAS responded better to treatment with added cetuximab when compared to patients without that drug, and that patients with mutated K-RAS responded very similarly to the FOLFOX-4 therapy, either alone or in combination with cetuximab. The authors have concluded that the K-RAS mutation status can be considered a highly predictive criterion in the decision of adding cetuximab to the FOLFOX-4 scheme for patients with untreated metastatic colorectal cancer.
CONCLUSION

Knowledge about colorectal cancer genetics has involved an increasing number of studies, which have led to the introduction of new paupaeudetic and therapeutic weapons to fight the disease. Nowadays, the use of target-drugs is a fact, as is the fact that new genetic tests have been proposed, such as the detection of microsatellite instability and DNA sequencing to define specific genetic mutations related to CRC. However, it is necessary to acknowledge that, despite the fact that the use of genomic studies is promising, there are complex limitations to be overcome, both in financial and logistic terms, before all that technology can be routinely used in clinical practice.

REFERENCE


