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REVIEW ARTICLE

Current trends in clinical genetics of colorectal cancer

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Summary

Recent innovations in molecular biology and colorectal cancer (CRC) genetics have facilitated the understanding of the pathogenesis of sporadic and hereditary CRC syndromes. The development of technology has enabled data collection for a number of genetic factors, which lead to understanding of the molecular mechanisms underlying CRC. The incidence and the nature of CRC is a mixture of genetic and environmental factors. The current field of interest is to understand how molecular basis could shape predisposition for developing CRC, disease progression and response to chemotherapy. In this article, we summarize new and developing genetic markers, and assess their clinical value for inherited and sporadic CRC.

Key words: *biomarkers, colorectal cancer, inherited syndromes, sporadic colon cancer*

Introduction

CRC is a major global health problem, with about one million newly diagnosed cases annualy. The highest standardized incidence rate (per 100,000 population) for CRC is in Australia and New Zealand (men-45.7, women-33), as well as in Western Europe (men-41.3, women-26.3). The lowest incidende is seen in North Africa (men-7.0; women-5.8), South and Central Asia (men-3.7; women-10.7). The incidence rate is typically slightly higher in men than in women (1.4:1). In comparison to other European countries, Serbia is on the nineteenth place in CRC incidence in men where the standardized incidence rate is 33.5 [1].

Sporadic, familial and hereditary are the three CRC forms. Sporadic CRC are the most common, and comprise about 70% of all CRC cases [2]. Familial forms follow, and are characterized with positive CRC family history, but the disease itself is not hereditary. Hereditary CRC has the lowest incidence. The incidence of familial and hereditary forms is estimated around 20-30%, and only 5% of these tumors have germ-line mutations [3]. Lynch syndrome (LS) and familial adenomatous polyposis (FAP) are the only CRC hereditary syndromes. LS have three phenotypic variants: Muir Torre syndrome (MTS), Turct syndrome, and constitutional deficits genes for DNA repair after replication [4]. FAP has two genetic variants: Gardner's syndrome and MYH-associated polyposis coli [5]. Hereditary intestinal polyposis syndrome commonly known as Peutz-Jeghers syndrome, is characterized by benign hamartomatous polyps in the gastrointestinal tract [6].

In this review, we focused on CRC clinical genetics, and how gene testing influences therapeutic management. Genetic markers are summarized with an emphasis on new and promising CRC markers for better outcomes in CRC management.

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Genetic markers – inherited colon cancer syndromes

Genetic tests are important in recognizing LS, while intensive screening for colonic and extracolonic malignances is required for mismatch repair (MMR) gene mutation carriers. The revised Bethesda guidelines (Table 1) [7], the MsPath model [8] and microsatellite instability (MSI) histology [3] are useful tools for screening LS. Germline mutations in MMR genes should be detected in LS diagnosis, therefore after initial patient selection based on clinical and histological criteria, genetic testing follows (MSI, immunohistochemistry for MMR proteins, MLH1 promoter methylation status, mutations of BRAF V600E). Immunohistochemical (IHC) analysis, is reliable to ascertain mutations in the MMR genes, however false negative results arise as certain "missense" mutations may functionally inactivate the correspondent MMR proteins (causing MSI), causing no changes in protein expression as detected by IHC [9]. IHC and MSI are recommended as complementary tests [10]. Microsatellite unstable CRC can be either sporadic or hereditary. Sporadic CRC are caused by random CRC inactivation of the MLH1 gene, mostly by promoter methylation (epigenetic changes), while hereditary CRC happen as a result of inherited mutations in one of the MMR genes or only sporadic CRC inactivation of the MLH1 gene [11-13]. CRC caused MSI are typically found in the right colon and are usually diagnosed at an early stage [11-13]. These tumors have a better prognosis, with varying response to 5-fluorouracil (5-FU) adjuvant chemotherapy [12,13]. This is especially true for CRC stage II and III, particularly when it comes to controversial responses in chemotherapy [14]. Specific genetic testing can help identify at-risk patients and their relatives. Inheritable diseases can be distinguished from sporadic CRC by using MSI tests with a *BRAF* mutation or *MLH1* promoter methylation add-on tests [15,16].

MTS consists of digestive neoplasms, typically CRC, accompanied by sebaceous neoplasms with or without keratoacanthoma. MTS is a rare phenotypic LS variation. Germline mutations in hMSH2 and hMSH6 genes cause MTS in 90% of the cases, while hMLH1 gene mutation is found in the remainder [14,17].

Turcts syndrome is a clinical entity characterised by primary brain tumors and CRC. This syndrome is usually caused by a germline adenomatosis polyposis coli (APC) gene mutation, seldom by MLH1, MSH6 and PMS2 mutations. Associations with FAP are also known [18,19].

A clinical diagnosis of FAP can be made if an individual has 100 or more adenomatous colon polyps diagnosed before the age of 40. FAP genetic basis is caused by germline mutations in the APC gene localized on the long arm of chromosome 5 (5q21). The APC gene is a tumor suppressor, first identified in 1987, and fully cloned in 1991. The APC gene product is a protein involved in signaling pathways, mitosis, adherence, migration, and apoptosis [20]. When the epithelium becomes multilayered, the APC gene is overexpressed in superficial cells, resulting in APC protein induced cellular apoptosis. Most APC gene mutations occur in the 5' end, and around 20% are localized in exon 15, while 3' end mutations are rare [20-22]. The degree of polyposis correlates with the localization of mutations. Mutations between codons 450-1600, specifically between codons 1250-1330, are associated with the development of more than 100 polyps (adenomas) [23]. Mutations starting at the proximal end to codon 158, as well as those at the distal end to codon 1900 are associated with a milder, attenuated variant of FAP (Attenuated adenomatous polyposis coli - AAPC). Mutations in exon 6 will result in AAPC, while mutations in the region between codons 157 and 168 in exon 4, also known as the boundary region, are crucial

Table 1. Revised Bethesda Guidelines (just one of these criteria need to be met)

B1 Individuals diagnosed with colorectal cancer before the age of 50 years
B2 Synchronous or metachronous colorectal or other HNPCC-related tumors (which include stomach, bladder, ureter, renal pelvis, biliary tract, brain (glioblastoma), sebaceous gland adenomas, keratoacanthomas and carcinoma of the small bowel), regardless of age
B3 Colorectal cancer with a high-microsatellite instability morphology* that was diagnosed before the age of 60 years
B4 Colorectal cancer with one or more first-degree relatives with colorectal cancer or other HNPCC-related tumors. One of the cancers must have been diagnosed before the age of 50 years (this includes adenoma, which must have been diagnosed before the age of 40 years)
B5 Colorectal cancer with two or more relatives with colorectal cancer or other HNPCC-related tumors, regardless of age

* Presence of tumor infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet ring differentiation, or medullary growth pattern [3]

irrespective of whether CRC will develop in AAPC or the classical FAP. Clinical diagnosis of attenuated familial adenomatous polyposis (AFAP) is suspected when an individual has between 10 and 99 adenomatous colon polyps, or more than 100 polyps diagnosed at an age older than the age expected for FAP (35–40 years or older). Mutations in exon 15 (codon 1309) are associated with an earlier disease onset and worse prognosis. APC gene mutations between codons 1445 and 1578 are associated with desmoids, osteomas, epidermoid cysts (extraintestinal manifestation of FAP) and polyps in the proximal gastrointestinal tract [22-24]. Moreover, it is known that germline mutations in the APC gene localized between codons 1250 and 1464 are associated with more than 100 polyps throughout the entire colon, and thus carry worse prognosis. Such prognosis may be due to more aggressive forms of the underlying disease or the presence of extraintestinal FAP manifestations. Exon 15 (between codons 1050 and 1464) has repetitive sequences prone to frequent "frame-shift" mutations that can result in stop-codons. In this region, a 5bp deletion (AAAAG) in codon 1309 causes the most common germline mutations. In around 20-50% of patients it is not possible to detect mutations in the APC (APC negative FAP) [23-25]. Genotype to phenotype correlation is a reasonable approach for screening, treatment and survival estimates. Localization of mutations in developed centers can influence the choice of surgical procedure. For FAP patients with mutations of codon 1250, the treatment of choice is total colectomy with an ileal pouch, as the carriers of these mutations may develop severe rectal polyposis [24,25]. Genetic sequencing of large regions, requiring greater material resources, is needed to identify these mutations.

MUTYH-associated polyposis (MAP) is an autosomal recessive, hereditary polyposis colon syndrome that primarily affects the ascending colon, and has a better prognosis than sporadic CRC [26]. MAP clinically resembles the attenuated FAP, however it often manifests FAP and LS phenotypes [26,27]. Adenomas occur in a small number in MAP patients and later in life, compared with the carriers of APC mutations. MAP patients have also increased risk for CRC in the range of 35-53% [26,27].

Clinical FAP surveillance

Screening recommendations include esophagogastroduodenoscopy (EGD), flexible sigmoidoscopy or colonoscopy every 1 to 3 years, starting between ages 10-15 [28]. If polyps are detected, endoscopic evaluation is necessary every 6 months to 3 years. If the number of polyps exceeds the capacity of endoscopic removal, a more invasive approach using colectomy is warranted. In the case of duodenal adenomas, repeated EGD every 1 to 3 years is warranted depending on endoscopy findings and clinical symptoms [28,29]. Therefore, annual physical exam that includes evaluation for extraintestinal manifestations and thyroid examination is necessary. In the case of AFAP, colonoscopy screening begins in late teens, and is repeated every 2 to 3 years, based on family history. Extraintestinal screening is similar to that for FAP. If a patients has a FAP/AFAP-like phenotype, but does not exhibit APC mutations, then MAP should be considered as a differential diagnosis [5,30].

Clinical LS surveillance

Current guidelines advise testing everyone younger than 70 years if positive for IHC/MSI. For patients older than 70 years of age, testing is required only for those who meet the revised Bethesda criteria [31,32]. LS patients have up to 30% risk for a second primary CRC in 10 years following initial diagnosis, therefore are recommended to have a colonoscopy every 1 to 2 years until the end of life or as deemed appropriate. LS patients and their blood relatives will need appropriate follow up if germline mutation affects one of the 4 MMR genes [31,32]. If these mutations are not detected or cannot be sequenced then the clinician must evaluate patient's risk of developing CRC and other cancers within the LS before proceeding with intensive endoscopic screening. Such a decision must be based on clinical, pathohistological findings, and molecular tests (e.g. hMLH1 promoter methylathion status, or BRAF). Clinical management of patients with MMR gene mutations reduces both the likelihood of the occurrence and mortality of CRC. It has been shown that the monitoring these patients reduces the risk of CRC and mortality by 60%. Families with LS and individuals at risk should start a monitoring program at 20-25 years, and reexam in 1-2 years [33]. Upper endoscopy is also recommended every 2 years, starting from 30-35 years of age, or 5 years before the onset of gastric cancer in the previous generation. According to one study [34], only 26% of patients had a positive family history, and most of them were more than 35 years old. There is no clear evidence of monitoring patients with LS in terms of early gastric and small bowel

Table 2. Clinical Lynch syndrome surveillance

Intervention	*Age (onset)*	*Interval (years)*
Colonoscopy	20-25*	1-2
Endometrial sampling	30-35*	Annual
Transvaginal US	30-35*	Annual
Urinanalysis with cytology	25-35*	Annual
Physical exam	21	Annual

*Or 10 years prior to earliest diagnosis in the family [38]

cancer [35]. Female LS patients are at higher risk for developing ovarian cancer, therefore annual gynecological exam, which consists of clinical examination and ultrasonography, is advised after 30 years of age [36]. Prophylactic hysterectomy with salpingoovariectomy should be considered in women with LS, especially if the patients are MSH6 mutation carriers and/or have positive family history of endometrial cancer [37,38]. Apart from gynecological malignancies, there is an increased risk of urinary tract cancer, which requires ultrasound examination and urine test annually. A recent study recommends urine cytologic examination for one year due to its non-invasiveness [38]. Screening for neoplasms which may occur within LS (associated with CRC, synchronous or metachronous) is not standardized and the decision should be based on the monitoring of the individual, in accordance with family history, as well as the clinical signs and symptoms (Table 2).

Thus, hereditary syndromes diagnosis requires careful clinical history and exam, as well as extensive genetic testing. Gene exams will depen on the patient history including family history asessessment. IHC/MSI is recommended for all newly diagnosed CRC patients.

Genetic markers – sporadic colon cancer

Cancer pathophysiology involves reactions of both proto-oncogenes activation and tumor suppressor genes inactivation before a cell becomes metaplastic. By identifying gene mutations or by altering gene expression, cancer genesis may not only be modified but it may also be individualized in the near future. For example, activating mutations, or the so-called gain-of-function mutations affecting KRAS, BRAF, and PI3KCA gene, shift cells to metaplasia via the MAPK chain of reactions leading to CRC genesis [39-41]. Thus, targeting ERK MAPK kinases is a very sensible approach as to alter the essence of cancer biochemistry. Other interesting therapeutic avenues, apart from RAF kinases, include MEK, the apoptosis signaling pathways, particularly the NF-κB, Bcl-2 and the TRAIL receptor.

KRAS proto-oncogene (Kirsten-RAS) is a message conveyor for the epidermal growth factor receptor (EGFR). KRAS gene most frequently mutates in codons 12 and 13 of exon 2, resulting in a protein that bypasses EGFR activities. KRAS mutations, which associate with approximately 40% of all CRC, occur early, and are typically caused by chromosomal instabilities. Chromosomal instabilities resulting in aneuploidy always carry worse prognosis than MSIs. Therefore, the European Committee on Health advises assessment of the KRAS mutation status for metastatic CRC before proceeding with biological treatments, particularly utilization of monoclonal antibodies targeting the EGFR [42].

Vascular support of neoplastic tissues is another important factor in both prognosis and therapy. Angiogenesis is a complex process guided by multiple factors. In the case of malignant tumors, the high mitotic index of neoplastic cells exceeds angiogenesis. The end result is hypoxic cells that translate the hypoxia-inducible factor-1 alpha (HIF-1-alpha) protein, a primary regulator of oxygen-dependent gene expression. HIF-1 alpha upregulates the vascular endothelial growth factor (VEGF) receptor (VEGFR) on cell surfaces, essentially leading to new vessel formation. Targeting overexpressed VEGFR in metastatic CRC with monoclonal agents is demonstrated to increase overall patient survival [43]. Therefore, standard chemotherapy and VEGFR blockade is the first line treatment in KRAS mutated metastatic CRC [44].

BRAF gene mutations make up 10% of all CRC with MSI. BRAF gene encodes a serine-threonine kinase that is a component of the RAS/RAF/MAPK pathway. When mutated, the kinase is upregulated and its out of control activity causes hyperplasia and inactivation of apoptosis. Exon 15 with the associated V600 mutation affects the majority of 80% of BRAF mutations, which negatively influence treatment outcomes for anti-EGFR monoclonal antibodies in metastatic CRC patients

Table 3. Specific pathways inhibitors of colorectal carcinogenesis

Drug	*Class*	*Target(s)*	*Patway(s)*	*Administration method*
Cetuximab [Erbitux]	Monoclonal Ab (chimeric)	EGFR	PI3K/Akt, MAPK	Intravenous
Panitumumab [Vectibix]	Monoclonal Ab (fully human)	EGFR	PI3K/Akt, MAPK	Intravenous
Bevacizumab [Avastin]	Monoclonal Ab	VEGF-A	Angiogenesis	Intravenous
Ziv-aflibercept [Zaltrap]	Recombinant fusion protein	VEGF-A, VEGF-B, PIGF-1, PIGF-2	Angiogenesis	Intravenous
Regorafenib [Stivarga]	Tyrosine kinase inhibitor	VEGFR1-3, PDGFR-β, FGFR-1, TIE2, KIT, RET, BRAF	Angiogenesis, tumor microenvironment, tumorigenesis	Oral

For abbreviations see text

with KRAS wild type tumors [42]. Vemurafenib is a novel enzyme inhibitor that blocks the B-Raf/MEK/ERK pathway by interrupting the B-Raf/MEK step. Although vemurafenib was shown as promising agent in treating melanomas, it may not necessarily be the case with CRC [45,46]. EGFR activation in CRC could explain why patients have a lower response to BRAF inhibitors [46,47]. Another point of BRAF gene is that BRAF mutation in microsatellite unstable CRC excludes LS [48].

Another key chromosomal site in terms of prognosis is 18q. It has been shown that somatic mutations have poorer prognosis in stage II and II CRC patients than patients having no altered 18q [49]. This is because 18q genes, e.g. SMAD2 and SMAD4, are important transduction pathways associated with the TGF-β pathway which plays a complex role in carcinogenesis. Loss of SMAD4 expression is associated with worse survival in multiple subsets of patients with CRC [49].

Current molecular CRC research resulted in the design of specific pathways inhibitors of carcinogenesis, approved at this stage only for metastatic CRC: EGFR inhibitors (Cetuximab [Erbitux], Panitumumab [Vectibix]), VEGF receptor tyrosine kinase inhibitors (Regorafenib [Stivarga]), anti-VEGF monoclonal antibodies (Bevacizumab [Avastin]), and VEGF Trap (ziv-aflibercept [Zaltrap]) (Table 3). Alternative target agents such as MEK, NF-κB, Bcl-2 and the TRAIL receptor are researched. Finally, identification of novel mutations, as well as differential gene expression, may become new targets in individualized therapy designs [44,50].

Novel genetic markers for colorectal cancer

Genetic research in CRC is a very unstable field with many exciting approaches that could open new avenues in CRC management. MSI and associated markers are intensively studied due to their significance in carcinogenesis. A general feeling exists that current chemotherapy protocols could be diversified to become more efficient by the addition of these new markers. For example, adding apoptosis inhibitors increases cell sensitivity to anticancer agents such as oxaliplatin and 5-FU in CRCs with unstable HSP110T17 [51]. The overall result is that roughly 25% of patients with stages II–III CRC with MSI have an excellent response to chemotherapy, due to large, biallelic deletions in the T17 intron repeat of *HSP110* in tumor DNA [51]. Germline polymorphisms assessment in the VEGF pathway may predict outcome in mCRC patients who undergo oxaliplatin/5-FU chemotherapy [52]. Some novel genetic markers can also address the negative effects of drugs, as shown in the case of adjuvant oxaliplatin and fluoropyrimidines toxicity in the phase III TOSCA trial in high-risk CRC [53].

Mediators of inflammatory response are also important agents in CRC research. Recently, IL-17A and IL-17RA were shown to activate ERK, p38 MAPK, and NF-κB, and promote proliferation of tumorigenic enterocytes that have lost the expression of APC tumor suppressor [54]. Women who carry a *BRCA1* or *BRCA2* mutation are at a high risk of breast and ovarian cancer, and may be at a moderate risk of other cancer types, specifically 5-fold increased risk of CRC among *BRCA1* mutation carriers younger than 50 years. Based on this evidence, women with *BRCA1* mutations should be counseled about their increased risk for early-onset CRC, and offered colonoscopy at 3-5-year intervals between 40 and 50 years. Women with a *BRCA2* mutation or older women should be managed as any other patient [55].

Aspirin has prompted new interest in the field of CRC. Aspirin was found to increase survival rates among patients with mutated-*PIK3CA* CRC, but not among patients with wild-type *PIK-3CA* cancer [55]. In another study, regular aspirin use was associated with a lower risk of *BRAF* wild-type CRC but not with *BRAF* mutated cancer. These findings suggest that *BRAF* mutant colon tumor cells may be less sensitive to the effect of aspirin [56].

Conclusion

Up to date there is no universal approach in treating all CRC patients, however the following steps provide good guidelines:

1) A more personalized therapeutic approach to suit the genetic encrypt is warranted in patients with *de novo* mutations.

2) The Amsterdam and Bethesda criteria are very useful in patients with inherited syndromes. Specific genetic testing may identify patients and relatives with elevated CRC risk.

3) Detection of mutated KRAS and BRAF reduces medical costs and improves patient outcomes using targeted therapies such as anti-EGFR monoclonal antibodies.

4) MSI as a marker for the efficacy of 5-FU can further help. Another microsatellite marker, i.e. HSP110, may provide useful information when chemotherapy is planned.

The clinical significance of numerous CRC oncogenes remains unclear. It may be the case that the role of these various yet increasing numbers of markers may require a more personalized approach in CRC management. The clinical significance of the numerous CRC oncogenes remains unclear. The rising number of novel CRC markers seems to shape a new landscape for more efficient personalized approaches to CRC management that will include the selection of patient-focused therapy and surveillance protocols.

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Conflict of interests

The authors declare no confict of interests.

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