COLORECTAL CANCER: A SMALL REVIEW OF CURRENT AND FUTURE MOLECULAR SCREENING MARKERS AND THE FIRST STEPS TO PERSONALIZED MEDICINE

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COLORECTAL CANCER: A SMALL REVIEW OF CURRENT AND FUTURE MOLECULAR SCREENING MARKERS AND THE FIRST STEPS TO PERSONALIZED MEDICINE (Abstract): Colorectal cancer is the third most common cancer, the fourth cause of death globally and represents 9.4% of all incident cancer in men and 10.1% in women, with high incidences reported in North America, Oceania and Europe, and low incidences in Asia and Africa. The improved prognosis in the past decades, and a 5-year survival rate which exceeds 65% in developed countries, demonstrate that colorectal cancer is a curable disease. Colorectal cancer is a very heterogeneous condition, in which early diagnosis and specific treatment project it in the curable area of disease, therefore a screening process must ensure effectiveness in reduction of disease mortality and cost. Serological markers have some limitations, but molecular markers, that explore chromosomal and microsatellite instability and mutations, are gaining ground to become the golden standard and will lead the way in new screening methods and personalized medicine guidelines. A major future challenge for all cancer biomarkers will be the integration of pharmaco-genomics, proteomics and metabolomics functional data, with epidemiological and clinical data. Keywords: COLORECTAL CANCER, MOLECULAR MARKERS, SINGLE NUCLEOTIDE POLYMORPHISM, PERSONALIZED MEDICINE.

Colorectal cancer is classified as the third most common cancer and the fourth cause of death globally, accounting for roughly 1 to 2 million new cases and 600,000 deaths per year in the USA (1-3). Although it's incidence is low at ages younger than 50 years, median age at diagnosis is about 70 years in developed countries (4). Worldwide colorectal cancer represents 9.4% of all incident cancer in men and 10.1% in women, however, it is not uniformly common throughout the globe with different populations experiencing different incidence rates, with high incidence reported in countries from North America, Oceania and Europe, and low incidence in countries of central Asia and Africa (5, 6). With improved, slow but steady, prognosis in the past decades, and a 5 year survival rate that exceeds 65% in developed countries, colorectal cancer is being seen as a curable disease with new molecular technologies used in prediction, diagnosis, and recurrence risk (4, 7-9). Unlike other types of cancer, no single risk factor can be pinpointed as having a high
prevalence, and a combination of multiple factors are highlighted in epidemiological studies. Of those cited we remind family history of colorectal cancer (10), diabetes (11), inflammatory bowel disease (12), smoking (13), excessive alcohol consumption (14), obesity (15), and high consumption of red and processed meat (16). The risk increase is strongest for people with first-degree relatives with colorectal cancer (those with relatives diagnosed at young ages or multiple affected relatives) and people with inflammatory bowel disease, and according to a large twin study, 34-35% of colorectal cancer risk may be attributed to heritable factors (17). Apart from hereditary forms, such as hereditary non-polyposis colon cancer like Lynch syndrome and familial adenomatous polyposis, which are understood as being determined by well-known genetic aberrations, that represent less than 5% of all colorectal cancer, genetic risk determining factors are still not yet understood (18). It is believed that the cause of colorectal cancer may lie in a cascade of genetic mutations that describes the adenoma-carcinoma sequence, in which normal epithelial mucosa gradually progresses to adenoma and then to carcinoma as a direct result of these changes (19, 20). Increased screening would most likely result in cost savings to the healthcare system, since more CRCs would be detected at an earlier stage and newer, more expensive chemotherapies could be avoided (21).

Molecular pathogenesis
Colorectal cancer molecular pathogenesis is very heterogeneous and it result is the loss of genomic stability, which needs to be understood if prognosis and treatment response are to be optimal (22-25). Several forms of genetic instability have been identified and include epigenetic gene instability, microsatellite instability (MSI), and chromosomal instability (CIN), of which the ladder includes 80-85% of all colorectal cancers through oncogene activation and loss of heterozygosity of tumor suppressor genes, and it is suggested that chromosomal instability induces carcinoma through the mutation or loss of the TP53 and APC genes, and activation of the KRAS, with a poor prognosis (19, 26, 27).

Microsatellite instability
Representing about 15% of CRC, microsatellite instability consists of deletions and insertions of short repetitive DNA sequences in a given gene, a consequence of DNA mismatch repair deficiency, where 3% of these are associated with Lynch syndrome and the other 12% are caused by sporadic, acquired hypermethylation of the promoter of the MLH1 gene, which occurs in tumors with the CpG island methylator phenotype (19, 28, 29). Although any single MSI in a gene may not have a significant subclinical or clinical impact, their accumulation can give rise to tumor progression. (19) They are frequently found in tumor suppressors such as activin receptor type 2 (83%) and transforming growth factor βR2 (69%) (19). MSI has a significant impact on tumor biology and is reflected by a more favorable prognosis of this molecular CRC subtype and a lacking response of MSI-H CRCs to 5’fluorouracil monotherapy (30). MSI status is determined in poorly differentiated cancers according to conventional histologic grading.

Epigenetic gene silencing
All stages of tumor formation to progression have been associated with chromatin alteration consisting in DNA methyla-
Cancers that present a great degree of DNA methylation can be considered CpG island methylation phenotype (CIMP) (31). In normal cells CpG islands are found in regions close to promoters, and if there is an aberrant hypermethylation tumor suppressor genes can be sliced repressing gene expression (19, 31, 32). Reversing gene silencing may be useful in cancer prevention and therapy.

**Genome-wide association studies**

Genome-wide association studies (GWAS) have evolved over the last years into a powerful tool for investigating the genetic architecture of human disease (33). They analyze and measure the variation in the genome's DNA sequence to identify genetic disease risk factors that are common in each population and rely on the identification of SNPs. The modern unit of genetic variation is the single nucleotide polymorphism or SNP. SNPs are single base-pair, high frequency occurring changes in the human DNA genomic sequence (34).

**SNPs**

They are best described as a variation at a single position in a DNA sequence of four nucleotide bases: A, C, G, and T. If more than 1% of a population does not carry the same nucleotide at a specific position in the DNA sequence, then this variation can be classified as a SNP (35). If a SNP occurs within a gene, then the gene is described as having more than one allele in which case, SNPs may lead to variations in the amino acid sequence. However, SNPs, are not just associated with genes as they can also occur in noncoding regions of DNA.

Most SNPs have no effect whatsoever on health and development, but some of these genetic differences, however, have proven to be quite important in the study of human health. SNPs may help predict the individual’s response to certain drugs, susceptibility to environmental factors, and risk of developing a particular disease. SNPs can also be used to track the inheritance of disease genes within families. Typically SNPs are used as markers of a genomic region, relatively even distribution in the human genome, with the large majority of them having a minimal impact on biological systems but can have functional consequences especially if they are found in coding regions (36-38). SNPs are increasingly becoming the DNA marker system of choice due to their prevalence in the genome and their ability to be used in highly multiplexed genotyping assays.

Identifying genes involved in the development of cancer is crucial to fully understand cancer biology, for developing novel therapeutics for cancer treatment and for providing methods for cancer prevention and early diagnosis. The use of polymorphic markers, in particular single nucleotide SNPs, promises to provide a comprehensive tool for analyzing the human genome and identifying those genes and genomic regions that contribute to the cancer phenotype (9, 39).

**Whole genome sequencing**

Today whole genome sequencing is the best method to obtain all an individual's genetic information, practically drawing a comprehensive genetic map, and even though the cost of sequencing is constantly falling, new technological advancements are required to make this method a cost-effective screening process.

**Clinical application of molecular markers**

Many CRC remain asymptomatic years
before they are diagnosed due to the time needed for the genetic mutation to take place. CRC has two gastrointestinal tumor markers used for screening carcinoembryonic antigen (CEA) and carbohydrate antigen (CA). Although elevated levels are associated with disease progression, they can be under the detection range until advanced disease stages are present, and post-operative they are important in disease recurrence.(40). Molecular markers have the advantage that they can be an early warning system not only raising awareness, but by their non-invasive nature be more appealing with a far greater compliance than standard screening methods such as colonoscopy and fecal occult blood test(FOBT). They can encourage more patients to undergo screening, and could significantly decrease CRC mortality.

**Current molecular markers used in diagnosis**

CIN markers include the RAS family inner surface plasma membrane protein encoders (HRAS, NRAS, KRAS) of which KRAS plays the leading role(26). It encodes GTP binding proteins that act like molecular switches, inactivating intracellular signal transducers for surface receptors such as EGFR (epidermal growth factor receptor), being an intracellular downstream component of the EGFR signaling cascade. Once activated the RAS gene suppresses apoptosis and promotes cell survival. From an oncogenic point of view the RAS family mutation is an early CRC event and as many as 37% of CRCs and 50% of adenomas larger than one centimeter exhibit this mutation(27). In 2008, exclusion of KRAS hot spot mutations in exon 2 has become mandatory for the application of EGFR-targeting antibodies in the first-line therapy of advanced colon cancer. The list of genetic alterations to be excluded for EGFR antibody therapy has been extended to KRAS exons 3,4 and NRAS exons 2–4 in 2013 after no responsiveness has also been found for these alterations(41). Other genetic changes like BRAF and PI3K mutations have been described to be associated with no, to some, EGFR antibody response, but the data generated in a different study is too controversial to include these markers in routine predictive molecular testing and therapy decision making(41-43). It is foreseeable, however, that the number of markers predicting therapy response in CRC will rise with new data being generated from larger CRC cohorts.

MSI markers identification relies on two main methods of detection that highlight genetic mechanism causing the disease, and are represented by polymerase chain reaction (PCR) and immunohistochemistry. MSI-H is a trademark alteration of hereditary nonpolyposis colon cancer (HNPCC)/Lynch syndrome-associated tumors, but is also found in sporadic colon cancers (44, 45). With the revised Bethesda guidelines in mind, colon cancer is tested for MSI to evaluate the possibility of Lynch syndrome (46). In combination with immunohistochemical analysis of the mismatch repair proteins (MLH1, MSH2, PMS2 and MSH6) and EPCAM40 the molecularly determined MSI status is the first step in genetic determination and counseling. Among the tested regions, when MSIs are present in two or more regions, the tumor is classified as MSI-High, otherwise, the tumor is considered as MSI-Low (MSI in one region) or MSI-Stable (no MSI) (28). Only in the absence of MSI-H, histological poor/high-grade differentiation (G3–4) is considered a prognostic marker while MSI-H cancers are considered low
Plasma methylated Septin 9 (SEPT9) DNA is a marker of colorectal cancer (3). SEPT9 gene encodes the Septin 9 protein, which is a member of GTP-binding proteins that involves in many cellular processes such as cell cycle (47, 48). Disruption of SEPT9 gene could result in tumor formation. In comparison with normal individuals the biomarker septin-9 has been found to be hyper methylated in nearly 100% of tissue neoplasia specimens and detected in circulating DNA fractions of CRC patients (49). In a study of 8000 patients that underwent routine screening colonoscopy, blood was collected and the results from the septin-9 test were compared to those of colonoscopy with a 67% CRC detection rate and 11% false positive rate (47). It is a sensitive screening test that can detect the majority of CRC, and can be combined with colonoscopy to ensure (50).

In the past few years GWAS have found some heterogeneity in colorectal cancer risk, and many polymorphisms have been showed to have a link in risk assessment, early diagnosis, evolution, and recurrence of CRC. As databases like SNPedia or DBsnp are continuously updating, there isn't yet a SNP or SNPs that can make up a screening panel that will have a good sensitivity and specificity. Although studies have shown an association between the presence of a certain SNP and CRC there is still little data that can standardize a single nucleotide polymorphism to a given condition, and with whole genome sequencing still at an excessive cost, modern technology is needed to ensure a cost-effective reliable method.

**CONCLUSIONS**

Colorectal cancer is a very heterogeneous condition in which early diagnosis and specific treatment project it in the curable area of disease, therefore a screening process must ensure effectiveness in reduction of disease mortality and cost. Serological markers like CEA and CA, have some limitation and in many cases unreliable in early detection, as well as imaging and invasive methods where the patient's compliance is key. Molecular markers that explore chromosomal and microsatellite instability as well as other mutations are steadily gaining ground and will most likely be the golden standard soon an open mind must be had and focused placed on molecular markers which show a great promise and will lead the way in new screening methods and personalized medicine guidelines.

Also, with every human genome sequenced and analyzed more and more information is gained and if the evolution of technology and cost is constant, whole genome sequencing will be the only screening test an individual will have to do. A major future challenge not only for CRC, but for cancer biomarkers in general will be the integration of pharmaco-genomics, proteomics and metabolomics functional data, with epidemiological and clinical data (51).

**REFERENCES**


