Biological and clinical markers in colorectal cancer: state of the art

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1. ABSTRACT

Colorectal cancer (CRC) is the World's third most common cancer. Its prognosis is closely related to the disease stage at the time of diagnosis. Here we review the role of clinical biomarkers (tissue, serum, and faecal) in the management of CRC. Molecular studies have recently widened the opportunity for testing new possible markers, but actually, only few markers can be recommended for practical use in clinic. In the next future the hope is to have a complete panel of clinical biomarkers to use in every setting of CRC disease, and at the same time: 1) to receive information about prognostic significance by their expression and 2) to be oriented in the choice of the adequate treatment.

2. INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the World. Each year, more than 300,000 new cases are diagnosed in the United States and Europe, with more than 940,000 cases worldwide and 500,000 persons die from the disease (1, 2).

The prognosis of patients with CRC is closely related to the disease stage at the time of diagnosis. Five-year survival is 90% in localized disease but lowers to 68% for disease with lymph node involvement. If distant metastases are present, survival drops to 10% (2). Many patients are diagnosed in an advanced stage, thus resulting in poor survival. Early detection of symptom-less CRC or its precursor lesions by population screening could reduce CRC mortality since removal of these precursors during colonoscopy reduces the incidence of CRC (4).

Since 50% of these cases develop metastases is obvious that aside other methods already well known for early diagnosis the need for easily available, performable and efficacious markers is highly felt (1,5). The aim is to early detect the cancer, predict prognosis, define therapeutic options, choose adjuvant or primary therapies, monitoring the response and detect recurrences

3. SCREENING SETTING

The several options for CRC screening follow into two broad categories: stool tests and structural exams. Stool tests include fecal occult blood tests (FOBt) and tests for exfoliated DNA. Structural exams include flexible sigmoidoscopy, total colonoscopy and computed tomographic colonographic/virtual colonoscopy (CTC). FOBt is the only screening method with a documented CRC mortality reduction in randomized controlled trials. The efficacy of strategies based on repeated guaiac FOBt has been established in three randomized trials and one non-randomized controlled trial (6-9). A meta-analysis after several rounds of these continuing trials showed a CRC mortality reduction of 14% during a 10-year screening period (10).

Colonoscopy is the only method that consent at the same time, diagnosis and primary prevention by detection and ablation of precancerous lesions (5). A recent study has showed a decrease of 67 % in the incidence of cancer respect expected cases on a cohort of 715 patients with a medium follow up of 14 years (11). The method is not free from risk, but, in general, the patients' compliance is acceptable. CTC and other radio-imaging techniques present some radio-protection issue and cannot detect smaller precancerous lesions (5).

Large screening programs proposing a test on blood, stool or urine sample have real chances to obtain full compliance. However, in our experience, population sensitivity to issues like cancer prevention is so high to overcome the fear of discomfort in case of more invasive procedures.

3.1. STOOL TESTS

3.1.1. *FOBt*

With this aim FOBt has been used for large mass screening and some recently conducted clinical trials have shown a significant reduction of mortality for CRC (8%) (6-10, 12). The most common and traditionally used FOBt are the guaiac-impregnated Hemoccult II (gFOBt) (Smith Kline Diagnostics) and Hemoccult II SENSA (Smith Kline Diagnostics). The guaiac blue color changes depend on the peroxidase-like activity of the heme/hemoglobin in stool. False positives can occur with dietary heme (red meat), plant peroxidases (certain fruits and vegetables) and secondary to upper GI bleeding due to consumption of aspirin and NSAIDs; false negatives with high dose of vitamin C. Another FOBt is based on the immunochemical (iFOBt) that works through antibodies that detect partial sequences of antigenic sites on the globin portion of human hemoglobin and do not require intact human hemoglobin for reactivity. These tests include HemeSelect (Smith Kline Diagnostics) and InSure (Enterix Inc.). These tests do not react with nonhuman hemoglobin or with plant peroxidases, making dietary restrictions unnecessary. The removal of dietary restrictions may potentially improve in CRC screening, as it did in one study from 53% to 66% (13). Because globin is degraded by upper GI enzymes, immunochemical tests do not detect blood from upper GI bleeding. A recent meta-analysis has showed a decrease of over 14 % in mortality with FOBt. In a recent pilot study, (conducted about 20,000 persons) asymptomatic individuals, aged 50-75, were invited to take part in one round of FOB testing and they were randomized to either a gFOBt or iFOBt. Primary goals of this implementation study were to determine the participation rate, feasibility and logistics of this type of screening. In this study, an overall participation rate of 53% was observed. Participation rate in iFOBt group was significantly higher than in the gFOBt group (60% vs. 47%) (14) Also, detection rates for advanced adenomas and cancer were higher for iFOBt therefore more colonoscopies had to be performed. Actually, other trials with iFOBt have been launched (15) since this has showed to be the most acceptable cost-effective way of performing large screening for CRC (16).

The FOBt present some advantages and disadvantages; A) advantages: 1) potentially examines the

entire colorectal tract, 2) non-invasive, 3) requires no patient preparation (unlike endoscopic investigations), 4) simple and affordable, 5) can be carried out in privacy of home, 6) most extensively validated screening test for CRC, and B) disadvantages: 1) low sensitivity for both adenomas (10%) and CRC (40-85%), 2) low specificity for both CRC and adenomas (90-98%), 3) the ingestion of certain foods and medicines can yield false-positive results with the guaiac-based assay, 4) multiple stool samples are necessary, 5) must be performed annually to increase chances of detecting intermittent bleeding. To improve sensitivity and specificity of screening new perspectives have been opened by molecular studies.

3.1.2. *Fecal DNA*

Faecal DNA tests detect mutant or abnormal DNA shed from neoplastic colorectal lesions and excreted in the stool. It is now clear that CRC, is preceded by multiples steps from a benign adenomatous lesion, (with various grades of dysplasia), and that each of these steps are due to different genetic mutations, many of these already known, has led to search for such alterations in stool. Since no single gene has been identified to be altered in all CRCs, a panel of DNA markers is usually employed. The most frequently measured markers in stool include mutant *k-ras,* mutant *APC*, mutant *p53*, *BAT-26* (long adenine tract 26) and long DNA (16).

Reviewing the literature, Hug and Brenner (17) concluded that DNA marker panels detected CRC with a specificity of 95% or greater. However, sensitivity varied from 60% to 90%. In 2004 the study by Imperiale (18) seemed to change in definitive way the approach to screening because the panel of faecal DNA (SDT 1) showed to be four times more sensitive and equally specific than iFOBT, detecting 51% of invasive CRC. The sensitivity decreased at 15% for advanced adenomas, while specificity remains high (94%) (18). Unfortunately this panel is more expensive and however less sensitive than colonoscopy and successive studies failed to demonstrate similar higher sensitivity respect to Hemoccult II (19,20) Among stool DNA test (SDT) we can identify two kinds of test: 1) SDT-1, included 21 tumour-specific point mutations (3 on the *k-ras* gene, 10 on the *APC*, and 8 on the *p53* gene); the microsatellite instability marker BAT-26; and long DNA, a marker for delayed apoptosis, which is characteristic of exfoliated neoplastic colonocytes (20); 2) SDT-2, sequence-specific DNA markers were detected by acrylamide gel electrophoresis, as described by Whitney *et al* (21); the panel consisted of 3 tumor-specific markers broadly informative for both CRC and adenomas (22): *k-ras* mutations, scanning of *APC* mutator cluster regions, and methylation of the vimentine gene.

Recently Ahlquist *et al* demonstrated that SDT-2 has significantly better neoplasm detection rates than did the FOBt or SDT-1 (20).

Actually, although tests based on a single gene have been proposed with important reduction of costs, these tests cannot be recommended for routine use in screening (23). The use of faecal DNA presents some advantages and disadvantages; A) advantages: 1) no restriction in diet or medication, 2) more accurate than FOBt, 3) may detect cancers of stomach and pancreas as well as CRC and 4) DNA is released continuously rather than intermittently via bleeding, thus obviating the need for multiple samples; B) disadvantages: 1) requires large volume of faecal sample, 2) expensive, may not be cost-effective, 3) laborious, 4) some genes, e.g. *k-ras*, may be mutated in normal-appearing colon, pancreatic hyperplasia and crypt foci cells and, 4) no evidence that screening with DNA markers reduces mortality from CRC at this stage.

3.1.3. *SFRP-2*

Nevertheless, the higher compliance respect endoscopy and presumed higher sensitivity respect to FOBT has led to further investigate on DNA markers in stool and in particular methylation (24) Wang *et al* demonstrated that the hypermethylated secreted frizzled-related protein 2 (SFRP-2) shows a strong correlation between expression in cancerous and precancerous lesions and presence in stool (25) SFRP2 gene was hypermethylated in 91.3% (63/69) CRC, 79.4% (27/34) and 53.8% (14/26) adenoma and hyperplastic polyp tissues, and in 87.0% (60/69), 61.8% (21/34) and 42.3% (11/26) of corresponding fecal samples. Most interesting is the high frequency in stool from patient with adenomatous lesion and the high specificity of SFRP that was not present in normal mucosa and only in two samples of faeces from controls. If these results will be confirmed SPFR can candidate as screening tool for early detection of cancer and premalignant lesions (25)

3.2. Serum and urine tests

Aside from tests on feces, many tests have been recently developed aimed to use humoral immunity against tumor associated antigens as possible biomarkers in early diagnosis of cancer. Unfortunately only few genes are available for this kind of search.

3.2.1 *p53*.

Encoded by TP53 gene, located on short arm of chromosome 17, p53 is a transcription factor and one of more investigated oncosoppressor gene. Many functions have been recognized for p53: repair of damaged DNA, block of cellular cycle until repair as been ultimated and promoting of apoptosis. Its alterations are frequent at early stage in natural history of many cancers. This is its force and its weakness since determines its lack of specificity.

3.2.2 *Others*

P53 is not the only gene able to raise an immunological reaction and against which serum antibodies have been found in serum: carcinoembryonic antigen, Ras, topoisomerase II-alpha, histone deacetylase 3 and 5, ubiquitin C-terminal hydrolase L3, tropomyosin and cyclin B1 have been studied an observed in serum of CRC patients. Though none of these genes is able alone to detect all cases of cancer, since each antibody is present only in a limited proportion of patients (usually < 40%), so appreciable results could be achieved only developing a panel comprising all these genes (signature) (27, 28).

3.2.3 *SELDI-TOF-MS.*

Proteomic technology based on a particular type of laser, Surface enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF-MS) and Protein-Chip arrays, can provide high-throughput protein profiling and has showed the ability to discriminate the serum of patients with a cancer and even the stage of cancer with high sensitivity and specificity and a total accuracy of over 75% up to 85% (29).

3.2.3.1 Alpha defensin

The alpha defensin 1, 2 and 3, produced in granulocytes, macrophages and Paneth cell of the small intestine share with the other members of family the antimicrobial effects but further enhance phagocytosis and increase the production of Tumor Necrosis Factor. The increase in serum of patients with different type of cancers has been related either with invasion of tumor by granulocyte, either with a direct production by tumor cells and this late opinion is actually predominant. Proteomic techniques can identify the alpha-defensin in serum of CRC patients with 100% of sensitivity and 69% of specificity. (30-32).

3.2.3.2 C3A anaphylatoxin

Complement-derived anaphylatoxin peptides, expressed in bronchial epithelium and smooth muscle cell in sepsis and asthma, have been demonstrated in serum of CRC patients with 97% sensitivity and 96% specificity, and in 86% of patients with colorectal adenomas. Although still not sufficiently validated, this marker appears very promising (30, 33).

3.2.3.3. Diacetylspermine

Diacetylspermine has been isolated in urine of patients with recurrent pancreato-biliary carcinoma, liver tumor, non small cell lung carcinoma and gynecologic malignancies. The same proteomic technology has been able to identify diacetylspermine in the urine of CRC patients with sensitivity for II stage CRC of 70% and a specificity of more than 90% (34).

Also this last marker need still of a validation on large numbers.

3.3-Hereditary cancers setting

Approximately 15% of CRC are thought to be due to an inherited or familial predisposition (35) The most common hereditary conditions giving rise to an increased risk of CRC are hereditary non-polyposis CRC (HNPCC) and familial adenomatous polyposis (FAP). In this particular high risk setting it is important to identify specific screening exams.

3.3.1 *FAP*

FAP is an autosomal dominant condition characterized by hundreds to thousands of adenomas in the colon and rectum. It has an incidence of approximately 1 per 8,000 to 1 per 14,000 of the population and accounts for about 0.5% of all CRC (36). CRC in an almost inevitable consequence of classical FAP, if untreated, with an average of onset of about 39 years of age.

An attenuated form of this syndrome (AFAP) is characterized by fewer adenomas (<100) Subjects with this attenuated form of FAP also have a high risk of developing CRC, i.e. approximately 80% by the age of 70 years. A further variant of the FAP syndrome results from biallelic inherited mutations in the BER. (base excision repair) gene, known as *MutYH* or *MYH* (37). This syndrome, which is now referred to as MAP or MYH-associated polyposis, is often indistinguishable in its clinical manifestation from classic or attenuated forms of FAP.

Approximately 70-80% of patients with classical FAP harbor germline mutations in the *APC* gene. Screening for FAP should commence with a detailed family history. For individuals with suspected FAP, genetic testing can be used both to confirm diagnosis in a suspected proband and to assess risk in pre-symptomatic family members. Provided the mutation responsible for FAP within a family is known, testing for *APC* mutations can be considered for at risk family members (38). Most expert panels recommend that for families with classic FAP, *APC* gene testing should be considered at 10-12 years of age (38, 39-41).

Moreover the surveillance of MIH should be considered in the 20-30% of families in which *APC* gene is negative (16).

3.3.2 *HNPCC*

HNPCC is clinically defined by the fulfillment of the Amsterdam Criteria (42) HNPCC includes affected families with disease causing mutations in DNA mis-match repair (MMR) genes displaying an MSI-H phenotype in their corresponding tumours (a subgroup also called Lynch syndrome) and families with MSS tumours and no mutations in DNA MMR genes. The genetic pathogenesis of the latter group is currently unclear.

Above screening families with diagnosis of HNPCC, the Bethesda guidelines revised criteria recommended a panel of 5 MS markers: 2 mononucleotides (BAT 25 and BAT 26) and 3 dinucleotides (D2S123, D5S346 and D17S250) (43). Tumours with no instability in any of these markers are considered to be MS stable (MSS). On the other hand, if one marker is mutated, the tumour is regarded to have low MSI (MSI-L) and if 2 or more markers are mutated, the tumour is regarded to have high MSI (MSI-H). The test should be performed on tumor tissue when HNPCC is suspected and, when confirmed, in the serum of consanguineous of the cancer subject. For diagnostic purposes, immunohistochemical tissue analyses with antibodies directed against MLH1, MSH2, MSH6, PMS2 and other MMR gene proteins may provide useful related information on MS status (36, 37, 40).

4. EVIDENT CANCER DISEASE SETTING (serum tumor markers)

Serum CEA and Ca 19.9 are commonly used as classical tumor markers in CRC patients. Ideal diagnostic markers must be characterized by both high sensitivity and high specificity. Presently, prostate-specific antigen (PSA) in prostate cancer is the most relevant diagnostic marker

approved by the Federal Drug Administration (sensitivity of PSA, positive rate of disease is about 80%; specificity of PSA, negative rate of non-disease is around 60%). In CRC patients, preoperative CEA and Ca 19.9 showed various degrees of sensitivity depending on the stage of disease, whereas specificity was as high as about 90%. The American Society for Clinical Oncology recommends that serum CEA and Ca 19.9 could not be used in the screening setting (44).

4.1. CEA

Preoperative setting. CEA may be performed preoperatively in patients with CRC if it would assist in staging and surgical treatment planning. Although elevated preoperative CEA (>5ng/mL) may correlate with poorer prognosis, data are insufficient to support the use of CEA to determinate whether to treat a patient with adjuvant therapy. Published data supports the utility of pre-operative CEA levels as prognostic factors. (45-47) Specifically, a study of 2,230 patients demonstrated that preoperative CEA was an important independent prognostic variable in predicting outcome (48); and another study of 1,146 CRC patients using a multivariate analysis confirmed that preoperative CEA levels was still a highly significant prognostic covariate even after stage and grade were included in the model (49) Preoperative levels of CEA are rarely elevated in patients with early cancer (0,5-10% of patients with TNM-stage 0-I and the frequency of positivity for this marker rises with the stage up to 78% in stage IV) High preoperative levels of CEA in III and IV stage CRC have been considered a strong independent risk factor for local recurrence and even for short DFS and OS (50-52).

Furthermore, determination of CEA before resection aids in assessing its utility for postoperative surveillance. An elevated preoperative CEA suggests that the marker would be useful for surveillance.

Postoperative setting. In the post-operative setting, serum CEA testing should be performed every 3 months in patients with stage II or III disease for at least 3 years after diagnosis if the patient is a candidate for surgery or systemic therapy. Elevated CEA levels warrant further evaluation for metastatic disease, but do not justify the institution or adjuvant therapy or systemic therapy for presumed metastatic disease.

Moreover, CEA is the most frequent indicator for recurrence in asymptomatic patients (44), is more cost-effective than radiology for the detection of potential curable recurrence (44), and is the most sensitive detector for liver metastases (44)

Metastatic setting. In metastatic CRC setting, CEA is the marker of choice for monitoring the disease during systemic therapy. CEA should be measured at baseline for metastatic disease and every 1 to 3 months during antiblastic treatment. Persistently rising values above baseline should prompt restaging, but suggests progressive disease even in the absence of corroborating radiographs. Caution should be used when interpreting a rising CEA level during the first 4 to 6 weeks of a new antiblastic treatment, since spurious early rises may occur

especially after Oxaliplatin use (53, 54)

Moreover, it is important to emphasize that measured levels of CEA may be different between laboratories and countries.

4.2. Ca 19-9

To date there are insufficient data to recommend the use of Ca 19.9 in the management of all steps of CRC (screening, diagnosis, staging, surveillance, or monitoring treatment of patients with CRC) (37, 44, 55, 56)

5. MOLECULAR STUDIES

Many possibilities have been explored aimed to identify patient at highest risk of recurrence and primarily of developing liver metastases. Actually , even after many molecular and genetic studies that have shown various relationship between genes and cancer behavior only few of these markers involved in tumor growth and metastatic processes have been validated as diagnostic or prognostic tool for use in clinical practice (57, 58).The genes and proteins can be divided based on their function

5-1 Adhesion

Adhesion is the mechanism that takes linked together the cells. When adhesion is broken, tumor cells are free to scatter and leave the site. On the other side, adhesion permits cells to bind to other proteins and colonize other sites.

5.1.1 *cadherin/catenin*

Among these proteins, E-cadherin and a-catenin have been demonstrated to be down-regulated in highly aggressive tumors (57, 59) The mechanism by which the complex is responsible for the formation of stable cellular junctions includes the link between cadherin and a submembranal beta catenin, between this last and a-catenin and to actin cytoskeleton (60). Free B-catenin translocates in nucleus where it acts as a transcription factor for proliferation genes.

5.1.2 *Mucins*

Mucins and especially MUC1 has been showed related with CRC. The mechanism seems include interaction and competition with binding of b-catenin by E-cadherin. This MUC1 binding to b-catenin is regulated by various proteins including glycogen synthase kinase-3ß, c-src tyrosine kinase, protein kinase C, and epidermal growth factor receptor (61). Mucins have been related with poor prognosis in sporadic CRCs, but not in hereditary cancers (62). In other series the better outcome of HNPCCs compared to sporadic CRCs seemed related to a lower prevalence of Mucins in the first group (63).

5.1.3 *Integrins.*

Integrins are a family of transmembrane glycoproteins that bind cells to extracellular matrix proteins of the basement membrane or to ligands on other cells as to laminin, collagen, fibronectin and vitronectin, conferring a particular aggressiveness to CRC in which they are highly expressed (57).

Table 1. Studies about multitarget stool dna testing for detection of CRC (da Ouyang)

Study	Panel	Sensitivity for CRC	Sensitivity for Adenoma	Specificity
Dong	p53, k-ras, APC	71%	NA	NA
Ahlquist	p53, K-ras, APC, BAT-26, long DNA	91%	82%	93%
Ahlquist	p53, APC, BAT-26, long DNA	91%	73%	100%
Brand	p53, K-ras, APC, BAT-26	69%	NA	NA
Tagore	p53, K-ras, APC, BAT-26, long DNA	64%	57%	96%
Syngal	p53, K-ras, APC, BAT-26, long DNA	68%	30%	NA
Calistri	p53, K-ras, APC, BAT-26, long DNA	74%	NA	97%
Imperiale	p53, K-ras, APC, BAT-26, long DNA	52%	15%	94%

5.1.4 *Osteopontine (OPN)*

Osteopontine or Secreted phosphoprotein 1 (SPP1), is a glycoprotein identified first in bone and successively in many tissues, i.e. brain, kidney, placenta, and in immune cells. OPN shows an anti-apoptotic role and interacts with integrins. OPN is over expressed not only in CRC but even in lung, breast, gastric, ovarian cancers, melanoma and mesothelioma (64).

5.1.5 *CD 44*

CD44 is glycoprotein. A cell surface glycoprotein binding hyaluronic acid and able to interact with OPN, collagen and matrix metalloproteinases. CD 44 is present in CRC and has also been found in prostate cancer, breast cancer and ovarian cancer.

Cell adhesion molecules may also mediate the selection of the host organ for the development of distant colorectal metastases. (63).

5.2. Invasion

Invasion is the most typical activity of tumor cells related with metastatization and is principally due to the action of some enzymes.

5.2.1. *Metalloproteases (MMPs)*

These zinc-dependent endopeptidases are thought to play a primary role in almost all cellular activities: proliferation, differentiation, dispersion, angiogenesis and apoptosis. MMP2 has been related with liver metastatization (65, 66).

5.2.2. Cathepsines

Cathepsin A (serine protease) and Cathepsin B (Cysteine protease), members of a numerous family of proteases, play a role in cellular turnover by breakdown of polypeptides, and have been related with progression of cancer (67, 68).

5.2.3. UPA *and PAI (Plasminogen activators (urokinase) its inhibitor)*

UPA is a component of the plasminogen activation system and, due to the extracellular matrix degradation following the activation of the proteolytic cascade, facilitates the invasion of tissues by cancer cells being a reliable prognostic factor (69). Further, UPA is a target for therapies since its inhibitors can act as targeted drugs.

5.3. Angiogenesis

5.3.1. *VEGF*

VEGF is the Vascular Endothelial Growth Factor, mainly derived from tumor cells, that determines blood vessel growth and indirectly promotes tumor growth and metastases by favoring tumor neo-vascularization. (VEGF) is a marker of poor prognosis in CRC. (70-72).

5.3.2 *Thymidine phosphorilases*

Thymidine phosphorilases a glycosilpeptidase involved in nucleotides metabolism, is a factor related with liver metastases (73).

5.3.3. *Inhibitors of angiogenesis*

Inhibitors of angiogenesis such as angiostatin, endostatin and thrombospondin-1 (TSP-1) can contribute to regulate the growth of liver metastases. These factors can be produced by primary or secondary tumors. Primary CRC producing such inhibitors, after its removal, can be followed by rapid growth of new vessels and then of metastases. On the other hands, in absence of inhibitors, synchronous metastases may be more frequent (57).

5.4. Cell growth

5.4.1 *Epidermal growth factor receptors (EGFR)*

Epidermal growth factor receptors (EGFR), member of the family of Erb- B receptors, are related with growth of cancer and, other than in breast cancer, have been reported to be highly expressed at protein level and more or less associate with gene amplification or mutation in 72% to 82% of metastatic CRC tissue samples (57, 74, 75). This marker is related to poor prognosis but can predict response to specific targeted therapy.

5.4.2 *K-ras.*

Activation, by single amino-acid change, of this proto-oncogene is one of the former steps in adenoma-carcinoma sequence and acts via activation of EGFR pathway independently from EGFR. This is why actually K ras is important in evaluating therapies since a mutant K-ras is a predictor of failure in response to anti-EGFR and should be routinely tested together with EGFR in all colorectal tumors (76, 77).

5.4.3 *APC*

APC is an oncosoppressor gene which encodes a protein binding the free b-catenin permitting its degradation and preventing translocation in nucleus. More than 800 possible mutations of this gene are already known and some

are responsible not only of FAP but even of many sporadic CRC. The test is useful in screening in individuals from family suspected to harboring the mutation. No prognostic value has been described for sporadic CRC (78).

5.5. Cell survival

Cell survival is linked to a balance between apoptotic and antiapoptotic factor. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is expressed by hepatic NK cells. Apoptosis can be triggered by the contact between TRAIL and its corresponding ligand as well as between tumor necrosis factor receptor FAS and its ligand FASL. The down regulation of the first and up regulation of the second can promote cancer progression and has been observed in liver metastases of CRC compared to primary tumors (79-81).It is possible that tumor cell escape to immunological aggression through desensitization to FAS/TRAIL killing. This mechanism can be enhanced by integrins and Src genes. This last gene codifies a tyrosine kinase and is highly activated in CRC (57).

6. CONCLUSIONS

Colorectal cancer represents a health problem in western Countries, with more than 940,000 cases worldwide. Its prognosis is closely related to disease stage. Above tumor markers in CRC the correct interpretation play a crucial role in the: screening, diagnosis and more recently also in the choice of target therapy. In the next future we hope to have an accurate panel of CRC tumor markers, especially in the screening and prognostic setting.

7. ACKNOWLEDGMENTS

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Abbreviations: CRC: colorectal cancers, FAP: familial adenomatous polyposis, HNPCC: Hereditary non polyposis colon cancer, APC: adenomatous polyposis coli , MSI: Microsatellite instability, FOBt: faecal occult blood test : , iFOBt: Istochemical faecal occult blood test , gFOBt: guaiac faecal occult blood test, OS: overall survival, DFS: disease free survival, EGFR: Epidermal Growth Factor receptor, TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand

Key Words: Colorectal Cancer, Tumor Markers, Screening, Prognosis, Target Therapy, Review

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