General Reports

Chirurgia (2012) 107: 555-563
No. 5, September - October

Prognostic and Predictive Factors in Colorectal Cancer

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Rezumat

Factori prognostici și predictivi în cancerul colorectal

Cancerul colorectal (CCR) este o problemă de sănătate publică; reprezintă a doua cauză majoră de mortalitate prin cancer în lumea industrializată, după cancerul de plămân: în fiecare an sunt înregistrate aproximativ un milion de cazuri noi de CCR şi 500 000 de decese datorate acestuia (1). Acestă analiză îşi propune să sintetizeze cei mai importanți markeri disponibili la momentul actual pentru CCR, care oferă informații prognostice sau predictive. Printre altele, abordează markeri serici precum CEA şi CA19-9, markeri exprimați de celulele tumorale, precum timidilat sintaza, şi de asemenea expresia sau deficitul de expresie al anumitor oncogene şi gene supresoare tumorale, precum K-ras şi p53. Valoarea predictivă a instabilității genomice, angiogeneza şi indicii de proliferare, precum indicele apoptotic, sunt discutate. Apariția unor noi strategii terapeutice a deschis calea unei abordări individuale a pacientului. Acesta va lua în considerație mecanismele genetice complexe implicate în geneza tumorală, adiacent stadializării clinicopatologice clasice. Numărul tot mai mare de agenți terapeutici şi de ținte moleculare în oncologie cunoscute determină imperiozitatea necesității studiului şi utilizării clinice ale markerilor biologici în vederea îmbunătățirii răspunsului la tratament şi a ratelor de supraviețuire, precum şi pentru reducerea toxicității şi instalarea unei stabilități economice. Potențialii markeri biologici predictivi şi prognostici descoperiți ca urmare a studiului bazei genetice şi a terapeuticii cancerului colorectal sunt de asemenea discutați.

Cuvinte cheie: carcinom colorectal, markeri prognostici, analiză, angiogeneză, instabilitate genomică, invazie, celule circulante

Abstract

Colorectal cancer (CRC) is an important public health problem; it is a leading cause of cancer mortality in the industrialized world, second to lung cancer: each year there are nearly one million new cases of CRC diagnosed worldwide and half a million deaths (1). This review aims to summarise the most important currently available markers for CRC that provide prognostic or predictive information. Amongst others, it covers serum markers such as CEA and CA19-9, markers expressed by tumour tissues, such as thymidylate synthase, and also the expression/loss of expression of certain oncogenes and tumour suppressor genes such as K-ras and p53. The prognostic value of genomic instability, angiogenesis and proliferative indices, such as the apoptotic index, are discussed. The advent of new therapies created the pathway for a personalized approach of the patient. This will take into consideration the complex genetic mechanisms involved in tumorigenesis, besides the classical clinical and pathological stagings. The growing number of therapeutic agents and known molecular targets in oncology lead to a compulsory study of the clinical use of biomarkers with role in improving response and survival, as well as in reducing toxicity and establishing economic stability. The potential predictive and prognostic biomarkers which have arisen from the study of the genetic basis of colorectal cancer and their therapeutical significance are discussed.

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Key words: colorectal carcinoma, prognostic markers, review, angiogenesis, genomic instability, invasion, circulating cells

Introduction

Colorectal cancer ranks among the most malignant diseases worldwide. It is a major cause of mortality, with a 5-year survival of approximately 50%. Metastasis to the liver and lungs are the main cause of death and occurs in up to 25% of patients at presentation. Histopathological factors such as tumour size, histological type and subtype, the presence of signet ring morphology and the degree of differentiation, as well as the presence of lymphovascular invasion and lymph node involvement are established factors that influence outcome. (2) Tumour budding, lymphocytic infiltration and resection margins have also been recognized for their prognostic significance. (3) However, understanding the molecular mechanisms underlying the metastatic process will help us to identify those at highest risk of recurrence and to find new tumour targets to prevent disease progression.

Prognostic and Predictive Biomarkers

Prognostic factor is defined as any parameter evaluated at diagnosis (or surgery), which is associated with treatment outcome (disease free interval, survival, local control).

Predictive factor is any parameter which evaluates the response or lack of response to specific treatment.

Over time, our experience in neoplasms led us to analyse many factors as prognostic and predictive factors.

Proliferation indices

Apoptotic index

The term "apoptosis" describes a process of programmed cell death clearly different from the other type of cell death, necrosis. Some of the main morphological characteristics of apoptosis are cell shrinkage, membrane blebbing, chromatin condensation, nuclear fragmentation and finally formation of apoptotic bodies (4,5). These morphological changes result from a vast series of cellular and biochemical processes triggered by physiological stimuli or activated in response to various forms of cell injury and stress (4,5). The balance between cell production through proliferation and cell loss through apoptosis determines how fast a tumour grows and is an important determinant of tumour behaviour. Most colorectal adenomas are stable for a long time before, if ever, transforming to malignancies MIB-1 (KI-67). The Ki-67 protein is a proliferation antigen, which is present in G1-, S-, G2- and M- phases of the cell cycle. Quiescent or resting cells in the G0- phase of the cell cycle do not express the Ki-67 antigen. Thus, determination of Ki-67 is an excellent correlate of the "growth fraction" of a population of cells, neoplastic or otherwise (6). Ki-67 is virtually restricted in its role as a proliferation antigen, making it a more specific determinant of growth fraction. Studies over the last decade have convincingly established the validity of assessing the Ki-67 antigen-expressing fraction ("MIB-1 labelling index") in tumours to indicate growth fraction, and correlated this value with a variety of clinico-pathological parameters (6).

Proliferating cell nuclear antigen (PCNA)

One of the first steps in multistage colonic carcinogenesis is increased cell proliferation. PCNA is known as a cyclin and an auxiliary factor in DNA polymerase. PCNA is the 36 KD polypeptide which is synthesized and expressed just in proliferating cells. The higher the PCNA expression, the higher the cell malignancy trend (7,8). PCNA plays a very important role in DNA replication (8). Because of this direct relationship with cell proliferation, PCNA is considered to be an important factor in prognosis. In fact, it has been described as a significant factor in the prognosis of CRC in several studies (9,10). The epidermal growth factor receptor (EGFR) pathway plays a substantial role in tumour growth through regulation of proliferation, angiogenesis, invasion and metastasis. It is mediated by downstream pathways including the RAS-RAF-mitogen activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR pathways. An increased understanding of the complex EGFR downstream signaling cascade and the importance of Kirsten rat sarcoma-2 virus oncogene (KRAS) mutation provided the starting point in identifying predictive biomarkers for EGFR directed therapies in patients with advanced colorectal cancer (ACRC). Therefore, mutations in KRAS can cause ongoing activation of the downstream RAS-RAF-MAPK and PI3K-AKT-mTOR pathways, whether the upstream EGFR is activated or not. KRAS mutations have been found in 35-45% of ACRC, most commonly in codons 12 and 13.

Serum markers

Carcinoembryonic antigen (CEA) is one of the first studied biomarkers in colorectal cancer.

Carcinoembryonic antigen (CEA)

CEA is a serum glycoprotein with a molecular weight of 180 kd that is one of at least 19 related molecules that are members of the immunoglobulin gene superfamily. As such, CEA functions as a homotypic intercellular adhesion molecule that promotes the aggregation of human colorectal carcinoma cells (11). CEA may facilitate metastasis of CRC cells to the liver and lung. CEA is a normal cell product that is overexpressed by adenocarcinomas, primarily of the colon, rectum, breast and lung (12). Smokers have a higher circulating CEA concentration than non-smokers, but there are no significant effects of age, sex, or ethnic group on the normal range. The liver is the major site for clearance of CEA. Moderate to significant elevations of serum CEA can be observed in a variety of chronic and acute inflammatory diseases, including alcoholic cirrhosis, cholelithiasis, obstructive jaundice, cholangitis, liver abscess, emphysema, bronchitis, gastric ulcer, gastritis, diverticulitis, diabetes and

collagen vascular diseases (13). CEA level elevations are not unique to CRC, but are observed in several carcinomas. The majority of the preoperative CEA studies showed that it was a useful prognostic indicator. As a postoperative prognostic indicator following complete surgical resection of colon carcinoma, elevated plasma CEA levels should return to normal within 4 to 6 weeks (14). An elevated postoperative CEA is an adverse prognostic indicator. Recently, CEA mRNA levels have also been found to be useful for the evaluation of CRC progression, with elevated post-operative CEA mRNA predicting the presence of micrometastasis (15).

CA 19-9

The CA 19-9 assay measures a tumour-related mucin that contains the sialylated Lewis - a pentasaccharide epitope, lacto-N-fucopentose II (14). CA 19-9 is produced by adenocarcinomas of the pancreas, stomach, gall bladder, colon, ovary and lung, and it is shed into the circulation. The upper limit of normal for healthy subjects has been defined by the cut-off value of 37.0 U/mL (17). CA 19-9 has become an established marker for pancreatic cancer, but it is still regarded as a research test for CRC. Numerous studies have addressed the potential utility of CA 19-9 in adenocarcinoma of the colon and rectum. The reported incidence of elevated serum CA 19-9 in CRC ranges from 20% to 40% (18). The incidence of elevated CA 19-9 is stage-related, with the highest sensitivity occurring in patients with metastases (19,20). However, the sensitivity of CA 19-9 was always less than that of the CEA test for all stages of disease (18,19). The false-positive rate (> 37.0 U/mL) is 15% to 30% in patients with non-neoplastic diseases of the pancreas, liver and biliary tract (20). Consequently, CA 19-9 cannot be used for screening asymptomatic populations. Significant post-surgical decreases are observed for CA 19-9, but these decreases have not been correlated with survival or disease-free interval. However, the CA 19-9 abnormality preceded clinical manifestation of the disease in only 25% of the cases and provided a median lead time of only 3 months (21,22). Serum CA 19-9 elevations may be observed in as many as 20% to 40% of patients with late-stage CRC, but cannot be regarded as a diagnostic aid nor can it be used to detect early stage disease. Progressive increases of the marker may signal disease progression in 25% of the patients who express the CA 19-9 marker, but this monitoring provides only a minimal lead time of 1 to 3 months. Monitoring with CA 19-9 has not been shown to improve the management of patients with CRC.

T-lymphocyte infiltration

T-lymphocyte infiltration in CRC patients is a suitable indicator of good prognosis. Four chemokine genes were chosen for further analysis. CD4+ and CD8+ T cell antigens or functionally related antigens, such as major histocompatibility molecules, interferon gamma reduced proteins and IL2RB were found in the co-expressed gene list. Findings suggested that lymphocytes form part of a tumour-specific host response implicated in minimizing the spread of cells from the primary tumour.

Biochemical markers

Thymidylate synthase (TS)

Thymidylate synthase plays an essential role in catalyzing the reductive methylation of deoxyuridylate (dUMP) to thymidylate (dTMP), which provides the sole intracellular de novo source of dTMP. Once synthesized, dTMP is then metabolized intracellularly to dTTP (the triphosphate form), an essential precursor for DNA biosynthesis. This reaction is critical as it maintains the essential metabolic requirements for cellular proliferation and growth. Because of its essential role in DNA replication, human TS is an anticancer drug target. TS is the target for the widely used anticancer agent 5-FU, which is active against solid tumours like breast, head and neck and colon cancers. TS has been suggested as a prognostic factor of survival in CRC (23,24) and of the response of tumour cells to 5-FU therapy where high TS levels correlated with a poorer prognosis. Increased TS levels in tumours are associated with resistance to chemotherapy with 5-FU. TS expression has been shown to be an independent prognostic factor in several cancers. Higher TS levels in hepatic metastases and resection margin are independent predictors of survival and progression in patients with metastatic CRC. Comparable results have been found in other tumour types such as gastric, (25) and cervical (25) cancers, with TS (+) tumours having significantly worse outcome compared to TS (-) cases.

Oncogenes / Tumour suppressor genes

p53

The p53 tumour-suppressor gene was also taken into consideration, its prognostic value being still under debate. Data suggest that its prognostic impact on sporadic CRC is dependent on p21 status. P53 is a tumour suppressor gene on the short arm of chromosome 17 encoding a protein that is important in the regulation of cell division. It is normally expressed when a cell senses DNA damage, producing a protein product that causes growth arrest and apoptosis (programmed cell death) in rapidly-dividing cells. In this way, p53 acts as a tumour-suppressor gene by aborting the growth of potentially malignant cells and/or established malignancies. Mutations of the p53 gene occur in various human tumours, including CRC (9,21). Deletions and mutations of the p53 gene can be detected in up to 85% of colorectal tumours and usually occur during the transition from adenoma to adenocarcinoma (26). Several functions have been ascribed to the p53 tumour suppressor gene, reviewed by Levine (9) and Sigal (10). Its product, the p53 protein, may respond to DNA damage by triggering either growth arrest during the G1- or G2- phase of the cell cycle or programmed cell death. In this manner, p53 may protect the normal cell from proceeding to replicate damaged DNA. A study by Valentini et al demonstrated higher p53 expression in colorectal tumours with microsatellite instability (27). The wild-type p53 protein, but not the mutant, can initiate apoptosis. The mutated p53 protein may block the function of the wild-type p53 protein

and thereby inhibit the induction of apoptosis. It has also been found that p53 has profound effects on responses to chemotherapeutic drugs used in CRC, and these effects vary considerably depending on the drug (28). Several studies have assessed the correlation between p53 protein expression and apoptosis in colorectal neoplasms. Some studies have indicated that adenomas and/or carcinomas with a high percentage of cells expressing the p53 protein were more likely to have a low apoptotic index (29-31), whereas most studies did not show such a relationship (32-37). In summary, the fact that neither the immunohistochemical overexpression of p53 nor p53 mutations correlated to the frequency of apoptotic cell death in the majority of studies does not support a major role for the mutant p53 protein as an inhibitor of apoptosis in the development of CRC (21).

Bcl-2

Bcl-2 is an intracellular membrane protein capable of inhibiting programmed cell death (37). The bcl-2 gene is overexpressed in follicular B-cell non-Hodgkin's lymphomas resulting from a t (38,39) translocation; however, overexpression of bcl-2 has also been detected in human epithelial tumours without translocation.At least 15 bcl-2 family member proteins have been identified in mammalian cells, including proteins that promote apoptosis and those that prevent it. The bcl-2 protein is normally expressed only in the lower half of the crypts of the colon, corresponding to the stem cell compartment, where bcl-2 is believed to protect stem cells from apoptosis. Most colonic adenomas express bcl-2 protein at high levels throughout the neoplastic epithelium, (29) while non- neoplastic polyps have a normal pattern of bcl-2 expression (40). Overexpression of bcl-2 may therefore contribute to the transition between hyperplastic epithelium and adenomas. The bcl-2 protein expression in CRCs is higher than in normal mucosa, but lower than in adenomas (32,40). An inverse correlation has been reported between bcl-2 expression and the apoptotic index of colonic tissues (29,32,41,42), whereas others have found no such correlation (31,33,35,36). With respect to the correlation between bcl-2 expression and prognosis in CRC, reports are conflicting (33,43,44). Considering the relationship between bcl-2 and p53 overexpression, some have found an inverse correlation in adenomas and carcinomas (42). Bcl-2 expression in poorly-differentiated clusters of cancer cells in the tumour growth zone has been found to be a probable factor determining the biological malignancy of CRC (45). Bcl-2 expression seems to be gradually reduced in the course of the adenoma-carcinoma sequence and inversely related to p53 overexpression. As most studies show a gradual increase in frequency of apoptotic cell death, a possible relationship with the down-regulation of bcl-2 can be hypothesised. However, bcl-2 is probably only one of the genes that determine the incidence of apoptotic cell death in colorectal neoplasms. Indeed, changes in the expression of other members of the bcl-2 family have been shown during the progression of colorectal tumours, such as the anti-apoptotic proteins bcl-XL, mcl-1 and the pro-apoptotic protein bak, which may be more important than bcl-2. Krajewska et al. showed that the expression of bcl-XL is increased in undifferentiated primary CRCs, often with accompanying reciprocal decreases in the anti-apoptotic proteins bcl-2 and mcl-1 and the pro-apoptotic protein Bak, whereas Bax expression is relatively constant. Thus, a shift from expression of the anti-apoptotic proteins bcl-2 and mcl-1 to the bcl-XL protein may occur during progression of colorectal tumours.

K-ras

The ras family of oncogenes comprises three principal members - K-ras, H-ras and N-ras - all of which have been implicated in the development of human malignancies (46). The K-ras oncogene located on chromosome 12p12 encodes a 21-kD protein (p21ras) involved in the G-protein signal transduction pathway, modulating cellular proliferation and differentiation (47). Mutations of the K-ras oncogene result in constitutive activation of this signal transduction pathway and, consequently, unregulated proliferation and impaired differentiation. Activating K-ras mutations are present in greater than 50% of colorectal adenomas and carcinomas and the vast majority occur at codon 12 of the oncogene (38). K-ras abnormalities are one of the earliest events in the stepwise progression of colorectal neoplasms, being detectable even in histologically unremarkable epithelium and aberrant crypt foci adjacent to cancers (26). The clinical significance of K-ras mutations is controversial, although some studies have shown lower median survival times in patients with mutation-positive tumours. Amongst other gastrointestinal malignancies, K-ras mutations are one of the most common genetic abnormalities in pancreatic and bile duct carcinomas, detectable in more than 75% of tumours (46,48). Measurement of ras oncogene abnormalities has not been demonstrated to be an independent predictor for either survival, quality of life, or disease-free survival in patients with large bowel carcinoma (39). Abnormalities of ras in colorectal tissue may correlate with increased relapse rate and decreased survival (48). However, currently available data do not demonstrate that ras oncogene analysis provides independent prognostic information in CRC.

Deleted in colorectal cancer (DCC)

The development of colonic carcinoma is associated with the mutation of a specific set of genes. One of these, named DCC (deleted in colon cancer), is a candidate tumour-suppressor gene (49). The contribution of molecular genetics to CRC has been largely restricted to relatively rare inherited tumours and to the detection of germ line mutations predisposing to these cancers. However, much is now known about the somatic events leading to CRC in general. Several studies have examined the relationship between genetic features and prognosis. Inactivation of the gene DCC located on chromosome 18 is known to be associated with the tumorigenesis and metastasis of CRC (50). Loss of heterozygosity (LOH) at the DCC gene locus was detected in colorectal tumours and this LOH is possibly related to metastasis. Saito et al. suggested that a decrease in DCC expression may play an

important role in the progression of CRCs and may be a biological marker of prognostic significance (51).

Genomic instability

Microsatellite instability (MSI)

Most cancers of the colon and rectum display a phenomenon termed genomic instability. There are apparently two distinct forms of genomic instability which reflect different genetic pathways of tumorigenesis. One form is observed at the nucleotide level, frequently resulting in deletions or insertions of a few nucleotides, and is termed microsatellite instability (MSI). It refers to a clonal change in the number of repeated DNA nucleotide units in microsatellites and appears in tumours with deficient mismatch repair (MMR) due to the inactivation of the four MMR genes: MSH2, MLH1, MSH6 and PMS2. MSI positive tumours are associated with a better prognosis in all stages of the disease. Microsatellites are polymorphic tandem repeats of short nucleotide sequences distributed through the genome. The inherent instability of microsatellite loci is primarily due to changes in the number of repeats during DNA replication as a result of inefficiencies in a proof-reading enzyme such as the mismatch-repair enzymes MLH1 and MSH2. Germline mutations in the genes that encode these enzymes are known to result in hereditary non-polyposis colorectal cancer (HNPCC) (52), but in addition somatic alterations in this group of genes can result in the observed high-level MSI that is found in 10-15% of colorectal tumours (53,54). Colorectal tumours that exhibit MSI surprisingly have a normal complement of chromosomes i.e. they retain a diploid karyotype (55). In contrast, however, about 80% of colorectal tumours are microsatellite stable, exhibiting a wide variation in chromosome number. This type of genomic instability has been termed chromosomal instability and, at the molecular level, is characterised by frequent cytogenetic abnormalities and allelic imbalance (AI), which represents losses or gains of defined chromosomal regions. This pathway of genetic instability is selected very early in tumorigenesis (56) and is possibly caused by the inappropriate chromosome segregation at mitosis that occurs when the APC gene is mutated, an event that occurs very early in most sporadic and hereditary colorectal tumours (38). MSI-positive tumours occur more frequently in the right side of the colon with approximately 75% of HNPCC and up to 90% of sporadically occurring MSI-positive cancers detected proximal to the splenic flexure (56). Survival data from several studies suggest that patients with HNPCC have a better prognosis than those with sporadic disease (53,58). The impact of 5-FU chemotherapy in 656 patients with Dukes' C CRC (median follow-up of 54 months) demonstrated a clear survival advantage (90 vs 35%) for MSI-positive disease. However, among patients who did receive fluorouracil-based adjuvant chemotherapy, high-frequency microsatellite instability was not correlated with increased overall survival. Little is known about how these two types of genetic instabilities might interact with specific therapies. However, even a small variation in the response of MSI-positive or -negative cancers to chemotherapy may have an important effect on clinical outcome. Clearly, more studies are required to assist in the choice of suitable chemo-therapeutic agents.

Angiogenesis

Recently, angiogenesis has gained increasing interest as a prognostic factor in a variety of solid tumours. The immuno-histochemical analysis of the newly-formed vessels is the most widely used index of angiogenesis in solid tumours (59). However, the levels of angiogenic factors have also been studied as a marker of angiogenesis and prognostic factor. Vascular endothelial growth factor (VEGF) is a glyco-protein similar to platelet- derived growth factor, and it is a potent angiogenic factor and one of the more widely studied as a prognostic factor in cancer patients (60-63), since it is considered to be the main angiogenic stimulator (64). Higher levels of VEGF are reported to correlate with the tumour burden and poor prognosis in patients with solid tumours (60,65). VEGF expression may also be induced by mutation of the p53 gene and activation of the ras/MAPK pathway in human CRC (66). Several growth factors have been identified that regulate angiogenesis in colon cancer; the most important of these is thought to be VEGF (67). VEGF is the major growth factor promoting angiogenesis in CRC. Overexpression can be identified in about 50% of cases. Most of the reported studies have suggested that VEGF expression is associated with an adverse prognosis (65,68). Evidence for the predictive role of VEGF with current available agents is lacking. However, in the future, tumours with high VEGF expression may be treated with targeted antibody therapy (45). The natural direct antagonist of VEGF is FLT-1, which may have a role in anti-angiogenesis therapy. It is detectable in the sera of cancer patients; however, its prognostic significance remains to be determined (69).

Markers of invasion / metastasis

Matrix metalloproteinases (MMPs)

To invade the surrounding tissue and metastasise, tumour cells need to secrete enzymes that will break down the components of the surrounding extracellular matrix (ECM). Such enzymes include the matrix metalloproteinases (MMPs), a family of neutral metalloenzymes that together are able to degrade all the components of the ECM. The MMPs are secreted as latent proenzymes. They require activation through proteolytic cleavage of the amino-terminal domain and their activity depends on the presence of Zn^{2+} and Ca^{2+} (70). Five MMP subclasses have been defined, grouped according to substrate specificity; interstitial collagenases (71), gelatinases, stromelysins, (72), metalloelastase (73) and membrane type-MMPs (MT-MMPs).MMPs are now known to contribute to multiple steps of tumour progression in addition to invasion, including tumour promotion, angiogenesis and the establish-ment and growth of metastatic lesions in distant organ sites. In addition, it is recognized that MMPs not only can be synthesized by tumour cells but are frequently produced by

surrounding stromal cells, including fibroblasts and infiltrating inflammatory cells. Finally, although creating gaps in matrix barriers remains a role for MMP activity, MMPs are also known to solubilize cell surface and matrix- bound factors that can then act in an autocrine or paracrine manner to influence cellular properties such as growth, death and migration. It has also been demonstrated that MMP and CD44 interactions are important in controlling tumour cell invasion and migration (74,75). In numerous work with different types of cancer, the expression levels of particular MMPs and their correlation with the clinicopathological characteristics of the patients have been studied. In general, there are 2 important aspects related to cancer progression in several studies: (i) the association between MMP expression and tumour grade or aggressiveness and (ii) the correlation of MMP expression and activity with recurrence or metastasis risk. Increased MMP activity and overexpression has been shown to correlate with tumour aggressiveness and metastatic potential in a wide range of cancers (70,72). Inhibition of MMP activity in the extracellular space has been extensively studied as an approach to inhibit growth and invasion of neoplastic cells. MMP inhibitors (MMPIs) have shown efficiency against malignant tumours in preclinical studies (76). MMPIs are currently being assessed in several phase II and III trials in the treatment of different cancers.

Circulating tumour cells

Circulating tumour cells of CRC have been of great interest in cancer research. The detection of circulating cancer cells in peripheral blood has become the primary goal of this research, whereas histological sections, lymph nodes and bone marrow were previously studied. New methods of detection include rare cell enrichment and detection techniques like fluorescence microscopy, flow cytometry, RT-PCR and methylated DNA PCR, of which RT-PCR is the most widely used molecular method for the detection of circulating tumour cells (77) Among the target genes for RT-PCR, CEA mRNA seems to be the most frequently screened for in the blood of patients with gastrointestinal carcinomas (77). A lot of different epithelial markers are targeted with these techniques e.g. cytokeratins, EGFR, CEA and EMA (77). Clinically, circulating tumour cells have been found to be independent prognostic factors in lymph nodes and bone marrow. In blood, their presence appears to be an early marker for recurrence and relapse (78).

Conclusions

The increase of knowledge in the field of molecular genetics has led to the identification of specific alterations present in the malignant evolution. Many of these have been proposed as biomarkers, which are of potential use in estimating CRC prognosis. Recently, there has been an impressive increase in the number of putative biomarkers capable of predicting the response to specific adjuvant treatment, but yet it is not clear whether they have prognostic value or therapeutic implications. They may well be useful in taking decisions regarding the prognosis of CRC patients, but further prospective trials are clearly required. Identifying and understanding molecular markers can improve the effectiveness of treatment in several ways; for example, it can lead to the development of marker-specific therapies. Prognostic markers may also improve the selection of adjuvant therapies by identifying those who will benefit most and therefore avoid toxic side effects of treatment in patients with the least risk for recurrence. For now, predictive markers remain an open question, but clearly they will have an important role to play in the future.

- Oncology is nowadays in a situation in which the huge stowage of data information on different levels of the neoplastic process requires their correlation and coherent fitting, in order to provide explanations and satisfying solutions with regards to taking the adequate therapeutic decisions.
- This situation might be described as a puzzle in which each piece from the large array available is still searching for its corresponding place in building the final picture.
- An approach in terms of prognostic and predictive factors is a valuable conceptual acquisition, which may clarify and open new directions in the avalanche of knowledge that defines colorectal cancer.
- Summarizing the previously presented data, we would like to highlight the biological markers which have captured nowadays scientists' attention as prospective predictive factors in colorectal cancer:
  - Cellular proliferation indices are in research today both in relation to the Ki-67 protein, which blocks proliferation, and to PCNA (proliferating cell nuclear antigen), the former being excellently correlated with the "growth fraction", and the latter can only be expressed by proliferating cells, being directly connected to the "aggressiveness" of the neoplastic process.
  - Serum markers have been considered as great hope both as early diagnosis means, possibly for screening, and as a prognostic factor. The CEA antigen, despite its lack of specificity, remains a useful prognostic factor in colonic cancer. Thus, its plasmatic levels should be restored to their normal values in a matter of 4-6 weeks after a radical resection.

    A19-9 has proved itself to be a specific marker in the development of colorectal cancer metastases. Its post-operative decrease has not been correlated with survival rates, and the screening of CA19-9 has not so far improved CRC patient management.

    T cell infiltration is an adequate good prognosis indicator in CRC, considering that these lymphocytes are part of an immune response of the host to the aggressiveness of the tumour.
  - Biochemical markers: Thymidylate synthase (TS) has been suggested as a prognostic and survival factor in CRC, as well as of the tumour cell response to 5-fluororacil therapy, meaning that its elevated levels are associated with resistance to this type of

chemotherapy.

- Oncogenes: The p53 suppressor gene is still in research, most studies being in search of a correlation between the p53 protein and the apoptotic index.

The RAS (K-ras, H-ras and N- ras) family of oncogenes, located on the 12p chromosome, codifies the 21 KD proteins involved in both the cell proliferation and differentiation.

The anomalies of this gene complex, which are present in tumour cells, can be connected to the high relapse and low survival rates.

The MMR system deficiency is responsible for the microsatellite instability phenomenon (MSI), associated with a better prognosis in all colorectal cancer stages.

VEGF is certainly associated with an unfavourable prognosis, while its predictive role in connection to the available drugs has not yet been demonstrated.

Research of the factors involved in tumour cell invasion and metastasis, meaning of matrix metalloproteinase (MMP), has demonstrated their direct correlation with metastatic aggressiveness and potential, and their inhibitors have proven their efficiency in preclinical trials.

Circulating tumour cells have proven themselves to be an independent prognostic factor. Meaning that their presence in the blood stream appears to be an early marker for recurrence and relapse.

Prognostic markers can improve patient selection for a given treatment, avoiding toxic side-effects in patients which are not included in the benefit group.

Prediction markers remain a subject of interest and debate of antitumour agent selection research.

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