Molecular Prognostic Factors in Rectal Cancer

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Abstract

The prognosis of patients with rectal cancer can be assessed mainly on the basis of clinical and pathological factors among which the pTNM stage is the most valuable indicator. Recently, different combinations of molecular markers, which have prognostic significance, have been identified in an attempt to establish a "molecular staging" that would permit – along with the histopathological staging – taking optimum therapeutic decisions in colorectal cancer. An example in this respect is the attempt to classify patients with rectal cancer stage II (N-) with a view to administering adjuvant treatment to the risk subgroup.

The prognostic role of various factors (biological, genetic, molecular, etc.) was classified into four categories according to their predictive value. This paper reviews the most recent research, especially regarding the factors in the third category in order to assess their impact upon prognosis and to identify the most valuable factors that could change the therapeutic algorithm of rectal cancers in the future. However, more statistical studies are necessary before these factors can become the basis of new therapeutic strategies or prognostic evaluations in rectal cancer.

Key words

Rectal cancer - prognosis - molecular markers - p53 - apoptosis - oncogenes

Rezumat

Aprecierea prognosticului pacienților cu cancer rectal se bazează în principal pe factorii clinicopatologici, stadiul pTNM fiind cel mai valoros indicator. Recent, diferite combinații de markeri moleculari au fost identificate ca având semnificație prognostică, în încercarea de a contura o "stadializare moleculară", care, alături de stadializarea histopatologică, să permită luarea unor decizii terapeutice optime în cancerele colorectale. Un exemplu în acest sens îl constituie încercarea de stratificare a pacienților cu cancer rectal stadiul II (N-) în vederea administrării tratamentului adjuvant la subgrupul cu risc.

Factorii de prognostic (biologici, genetici, moleculari, etc.) au fost clasificați în patru categorii, în funcție de valoarea lor predictivă. In articolul de față se realizează o trecere în revistă a ultimelor cercetări , în special cu privire la factorii de categoria a III-a, în încercarea de a evalua cât mai bine impactul acestora asupra prognosticului şi de a identifica cei mai promițători factori ce ar putea schimba pe viitor algoritmul terapeutic în cancerele rectale.

Mai sunt însă necesare studii statistice solide care să evalueze aceşti factori înainte ca aceştia să poată constitui fundamentul unor noi strategii terapeutice sau estimări prognostice în cancerul rectal.

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Introduction

Rectal cancer is a frequently encountered lethal disease. Each year 42,000 new cases are diagnosed in the United States. The main treatment is surgery. Pre- or postoperative adjuvant therapy has been used in an attempt to improve the results of surgery in rectal cancer cases.

Prognosis

Many studies regarding prognostic factors in rectal cancer – many of which are predictive factors for treatment - have been carried out. The Consensus Conference regarding prognostic factors took place in 1999 and defined the following categories (1):

Category I – factors whose prognostic value was established by means of statistical records of multiple clinical trials and that had been used in patient management. This category includes: local extension of the tumour, regional lymph node invasion, micrometastases of the lymph nodes, vascular invasion, residual tumour and level of serum carcinoembryonic antigen (CEA).

1. Local extension of the tumour and regional lymph node invasion is expressed by means of the TNM system; survival at five years differs: stage I – 72%, stage II – 52%, stage III – 37%, stage IV – 4% (2).

2. Nodal micrometastases are defined as tumoral foci of < 0.2 mm which are identified histologically (code pN1) or by non-histological methods: polymerase chain reaction (PCR), immunohistochemistry (IHC) to identify cytokeratin, epithelial membrane antigen, carcinoembryonic antigen or specific tumoral RNA (code pN0).

If the results of immunohistochemical methods are also taken into account, a superstaging of up to 50% of the cases can be reached, however, the prognostic significance of micrometastases has not been validated in all studies (3). Although their presence proves the potential of the tumour to disseminate distally, they can be considered pN1 only to the extent to which they have a certain size and if they produce a stromal reaction with its own neovascularisation (4).

3. Vascular invasion of unmuscularised veins or small vessels influences the local and distal recurrence. Tumour cells must be detected histologically beneath the endothelium (5). The sensitivity of histological methods can be increased by using immunohistochemical techniques.

4. The residual tumour is defined by the terms R1 and R2 indicating the microscopic and macroscopic residual tumour, respectively (1).

5. Serum CEA. Patients with negative lymph nodes who have an increased preoperative serum level of CEA must be considered at a higher risk of recurrence after surgical treatment without adjuvant therapy (5,6).

Category IIA includes intensively studied biological and/or clinical factors whose prognostic and/or predictive value for response to treatment has been proven. The importance of these factors is high enough for them to be included in the pathological anatomy report, but thorough statistical studies should be carried out in order to validate them. This category includes tumour grading, resection margins and residual tumour after neoadjuvant treatment.

1. *Tumour grading.* It reflects the degree of tumour differentiation. Its evaluation is subjective since there is interobserver variability. It is recommended to evaluate the grading according to glandular formation (as the sole criterion) (1).

2. *Circumferential resection margins.* In rectal cancer the circumferential resection margins are represented by the whole exterior surface of the resected part. They are considered positive if the tumour is at < 1 mm from the surface. Since they are an important predictive factor for the local and distal recurrence, the positive resection margins represent an indication for adjuvant treatment (7).

3. *Residual tumour following neoadjuvant therapy.* Starting with the year 2002, the American Joint Committee of Cancer (AJCC) has introduced the substage ypTNM for patients who received neoadjuvant treatment. This differentiation will allow for the collection of data necessary in order to evaluate the results of neoadjuvant therapy.

Category IIB includes prognostic factors for which there are not sufficient data to include them in either category I or IIA.

1. *The histological type* – with the exception of undifferentiated types with "seal ring" cells – has not proved to be an independent prognostic factor .

2. *Microsatellite instability.* It refers to the insertion or deletion of the short repetitive DNA sequences, which can lead to DNA expansion or contraction. Colorectal cancers (CRC) with a high level of microsatellite instability (defined as instability in > 30% of the microsatellite loci) have several histological characteristics: they are more frequently located on the right colon, they are often of mucinous type and contain lymphocyte infiltrate (which is considered a factor of favourable prognosis by some studies) (8). Other studies have shown that microsatellite instability is very low in rectal cancers occurring in families at risk for HNPCC (9).

3. *18q deletions*. On chromosome 18 there are three areas involved in the late phases of colorectal tumorigenesis: DCC, SMAD4 and SMAD2. Among these, gene DCC (Deleted in Colon Cancer) has been studied more carefully. It encodes a transmembrane surface molecule similar to adhesion molecules. It is involved in the intercellular interaction playing a role in metastasis formation, it induces apoptosis and cessation of the G2M cellular cycle in the colorectal cancer cells. The loss of 18q heterozygosity (18qLOH) has been reported as an independent prognostic marker in CRC and it has also been associated with the presence of metastases (10).

Not all the studies sustain the role of DCC in tumour genesis because its expression is similar in normal, tumoral and metastasis tissue (11). At the same time, its prognostic role has not been proven in some studies (12).

Survival at five years in colorectal cancer stage II is significantly lower in patients with deletion of the 18q allele than in those without 18q LOH (54% as to 93%) and therefore the prognosis of patients in stage II with 18q LOH is similar to that of patients in stage III, whereas the patients in stage II without 18qLOH have a similar survival rate to the patients in stage I. 18q deletion also leads to a decrease in five-year survival in stage III patients (38% as to 52%). These data led to the conclusion that in patients with negative lymph nodes (stage II) the 18q loss represents an indication for adjuvant treatment (13,14).

In a study including exclusively patients with curatively resected rectal cancer without local recurrence but with distal metastases, the lack of DCC expression was correlated with a lower survival rate; however, the positive predictive value was not sufficient (19%) for the metachronous metastases that occurred in stage II patients. The conclusion of the study was that – in rectal cancer – the lack of DCC expression does not account for adjuvant treatment in stage II of the disease (15). The prognostic role of the loss of heterozygosity or of the lack of DCC expression in rectal cancer was not proven in some studies (16).

4. Tumor border

In several multivariate analyses the irregular infiltrating tumour border is a negative prognostic factor irrespective of the disease stage; it can also have a predictive value for metastases. It can be evaluated macroscopically when analyzing the resected piece or microscopically when the tumour goes beyond its own muscularis without any response from the stromal tissue and/or when small groups of malignant cells or glands pass into the adipose tissue of the mesentery or when there is perineural invasion. The presence of perineural invasion is a negative prognostic factor after neoadjuvant therapy (17). The quantification of the perineural invasion according to the depth and number of malignant cell foci per field is associated with local recurrence and five-year survival and it is independent of the T or N stage (18).

The concentration of undifferentiated cells in front of the tumour invasion area – "tumour budding" – is a negative predictive factor for rectal neoplasms . It can be found both in well and in moderately differentiated tumours (19). For the T1 or T2 tumours treated by transanal local excision it is predictive of local recurrence; therefore, it is an indication for completing the surgical treatment with other therapeutic methods (20). Its presence can be associated with the myxoid immature stroma in the structure of rectal cancer and it represents a negative prognostic factor (21).

Category III – includes factors that have not been sufficiently studied to establish their prognostic value.

1. Genetic markers

In colorectal cancer, it is considered that some molecular phenotypes – including certain molecular markers, which are independent of the pathological stage – are associated with more aggressive types of tumours. In the future the CRC molecular phenotype will be used in order to establish the molecular stage and to help in taking therapeutic decisions, especially for adjuvant therapy in patients of the stage I, II and III subgroups.

If a molecular marker can provide the same information as the stage or the component parts of a stage (e.g., pN), it can be useful as a prognostic factor even if it is not independent of the stage. These molecular markers can be evaluated at the initial bioptic diagnosis and they can indicate the aggressivity of the tumour before resecting it and examining it histopathologically. Thus, it can contribute to establishing the indication for immunotherapy and for the new genetic therapies.

The most valuable prognostic molecular factors are those indicating tumour aggressivity irrespective of the stage. These tumour markers are probably more closely related to tumour biology than to intraoperatively established tumour extension. An example in this respect is represented by the tumours with microsatellite instability that have a less aggressive clinical evolution even if they are large.

In order to use a protein as a prognostic marker, it must fulfill three criteria:

- to offer independent information which is even better than that of histopathological markers;
- to provide information that can change therapeutic decisions;
- the studies evaluating its prognostic value should be reproducible (22).

A. Genes influencing apoptosis

The p53 gene located on the short arm of chromosome 17 is a suppressor gene regulating cell progression from phase G1 to phase S in the cellular cycle. It regulates epithelial homeostasis either by inducing apoptosis (programmed cell death) or by stopping the cellular cycle in case of DNA defects (caused, for example, by exposure to radiation). In normal tissue the expression of protein p53 is low because the wild type of p53 has a short half-time (20 minutes) and cannot be detected by means of standard immunohistochemistry (IHC). The protein coded by the mutant p53 gene is stable and can be detected immunohistochemically. Therefore, if p53 is detected in a tumour, it means that it is the mutant type (23).

In rectal cancer p53 can be detected in 39-60% of the cases (23,24), while in colorectal cancer it can be found in 30-76% of the cases (25). It occurs more rarely in differentiated mucinous tumours and in tumours located on the right and transverse colon. Some studies proved the independent prognostic role of the nuclear accumulation on p53 upon survival and local recurrence (23). Few studies regarding rectal cancer reported that the presence of the p53 mutation negatively influences the five-year survival (24).

In a group of 66 patients with low rectal cancers (most of them Duke's C) treated only surgically, tumour recurrence was more frequent in patients with negative p53 (26). However, in this study the local recurrence rate was 64%, much higher than usually, even higher than in the studies concerning advanced rectal cancers – which is a possible factor influencing the obtained results. There are studies in which the researchers found a decrease in the mean period of time up to the occurrence of local relapse and a higher rate of local recurrence in patients with p53+; this suggests that the preoperative evaluation of p53 mutations could change the surgical strategy (27).

The relationship between the neoadjuvant treatment and the presence of p53 in rectal tumours was analysed in the Swedish Rectal Cancer Trial (28). The p53+ patients did not benefit from the preoperative RT whereas in the p53- ones there was a significant decrease in the local recurrence rate. The following mechanism accounts for this finding: due to the fact that the DNA is affected by radiotherapy (RT), apoptosis is induced in the cells with intact p53 function (IHC p53-), but this effect is limited in the cells with reduced p53 function (IHC p53+). At the same time, the response of p53+ tumours to preoperative chemotherapy is also low (29). The results of the studies regarding the predictive role of p53 in the local response to preoperative adjuvant therapy in advanced stages of rectal cancers differ considerably (24,30). The presence of p53+ can be associated with a decrease in the complete pathological response to preoperative chemo- and radiotherapy (RCT).

In the patients who had postoperative RT, the p53 rectal tumours recurred locally more frequently but this fact has not been confirmed in the multivariate analysis (31).

When ploidy was also taken into account as a molecular prognostic factor in primary and recurrent rectal tumours, p53 expression was higher in the group with a shorter disease-free interval and it could be correlated with aneuploidy and early local recurrence (32).

Since the standardization of IHC techniques caused numerous problems, more specific tests were used to evaluate the mutations of p53 gene in colorectal cancer – either indirectly by single-strand conformation polymorphism (SSCP) or directly by gene sequencing. By using gene sequencing in colorectal cancer an association of the p53 gene with distal location and with a lower survival rate was found. Using SSCP, other authors showed that p53+ is more frequent in patients with CRC N+ (32), whereas patients with stage III p53+ CRC have a lower survival rate; this fact was not, however, confirmed by all studies (33). Survival in rectal cancer was not influenced by the p53 mutation (detected by means of these genetic techniques) (34).

The data available at present do not indicate whether it is convenient to change the surgical strategy only on the basis of the intratumoral p53 detection. The role of p53 mutation in rectal cancer is still unclear; in colorectal cancers its role is controversial (25) since some authors consider that it plays a role in resistance to radiotherapy (24).

The literature is unclear about the prognostic importance of p53 because:

- p53 is expressed differently in populations of various ethnicity;
- tumour location differs;
- the method of nuclear accumulation analysis and evaluation can vary (evaluation limit, antigenic coverage methods used before immunostaining) (35).

As compared with colonic cancer, the p53 mutation is more frequent in the pathogenesis of rectal cancer along with the beta-catenine pathway (36) whereas microsatellite instability and the mutations of APC and K-ras genes are more rare (37).

Bcl-2 is a proto-oncogene blocking apoptosis and prolonging the life of certain cells. It is involved in malignant haematological diseases but also in epithelial tumours. The combination p53+/bcl-2- has a negative prognostic role (lower survival rate and shorter disease-free interval) in all Caucasian patients with CRC irrespective of its location (35).

Patients with high levels of bcl-2 have a good clinical prognosis; this fact was not confirmed by all the studies. The lack of bcl-2 expression was correlated with local invasion, metastases and local recurrence by some authors (38), a fact refuted by other authors (39). A meta-analysis performed in 9 studies including 1, 989 patients showed that bcl-2 was a useful prognostic marker in CRC (35).

In rectal cancer bcl-2 is present in 29% of the patients with curative resection and is associated with a longer disease-free survival and a lower local recurrence rate. However, the prognostic importance of bcl-2 is lower than that of p53 (40); at the same time, its prognostic role was not confirmed by all the studies .

Survivine is a recently discovered apoptosis inhibitor. It can indicate the disease-free survival after preoperative RCT and surgical treatment (41).

B. Oncogenes

K-ras. The ras gene family consists of three homologues k, h, n ras coding similar proteins of 21kDa. It is considered that the ras membrane protein plays a main role in translating proliferative signals. Ras-activating mutations – especially the punctiform ones in the k-ras gene codons 12 and 13 were detected in 20-43% of the CRC cases. It is involved early in the adenoma – carcinoma sequence and has an independent prognostic role. Only some studies with k-ras and p53 mutations confirmed the unfavourable clinical evolution.

The RASCAL study carried out in 2,721 patients with colorectal cancer indicated the existence of an independent prognostic effect, the mutation of codon 12 being particularly associated with a poor prognosis (42).

The mutation of the k-ras gene is more frequent in the cancer of the colon than in the rectal one (43). The presence of the c-K-ras mutation indicates a better tumoral response in advanced rectal cancers undergoing preoperative RCT (44). The combination of p53 and K-ras mutations represents a stronger negative prognostic factor for rectal cancers than p53 alone (27).

C-myc – with a role in tumour initiation and progression – is transcriptionally activated by beta-catenine; they are both expressed in CRC with serous invasion and lymph node involvement (22). The multivariate analysis indicates a worse prognosis for colorectal cancer patients with p53, bcl-2, c-myc expression than for patients in whom only one of these oncoproteins is expressed (39). The role of the c-myc gene in the prognosis of neoplasms located strictly in the rectal area has not been evaluated yet.

C. Genes related to DNA synthesis

The thymidylate synthase gene – located on the short arm of chromosome 18 – includes seven exons and encodes a protein containing two subunits. This protein catalyses the conversion of 2' deoxyuridine – 5'monophosphate to deoxythymidine monophosphate – a reaction which is essential for DNA synthesis.

The thymidylate synthase (TS) covalently binds 5-Fluorodeoxyuridine – the metabolite of 5-Fluorouracil – thus inhibiting the S phase of the cellular cycle. This effect is visible only when continuous perfusions of 5-FU are administered. If there are high levels of TS, resistence to 5-FU develops. There are studies proving that TS expression is higher in the primary tumour than in distal metastases and in lung metastases (which have a higher resistance to chemotherapy) than in liver metastases, which accounts for the poor response to 5-FU (45).

Approximately 65-69% of the rectal cancers are TS positive (46). Lack of TS positivity can predict down-staging in advanced rectal tumours preoperatively treated with radiochemotherapy but it does not correlate with survival (46,47). In terms of local recurrence, distal metastases, disease-free survival and overall survival, the patients with a low intratumoral level of TS have a more favourable prognosis than those with higher levels of TS.

In rectal cancer TS is associated with a more intense proliferative activity expressed by Ki-67. In a comparative study including p53 and bcl-2, TS proved to be the best predictor of sensitivity or resistance to preoperative RCT in patients with advanced rectal cancer (47).

D. Genes inhibiting cycline-dependent kinases

In the cellular cycle the transition from phase G1 to phase S is regulated by major factors such as cycline-D and cycline-dependent kinases CDK 4 or 6. The complexes formed between cycline-D and CDK4 or 6 are triggered by the activating CDK kinase leading to hyperphosphorylation and inhibition of the retinoblastoma gene Rb – having a tumour suppressing role.

The WAF1/CIP1 (p21, p27, p57) and INK (p 15, p16, p18, p19) families of CDK inhibitors (CKIs) prevent progression in phase S by negative feedback upon the CDK-cycline complexes.

Besides inhibiting CDK activity, p21 can bind the cellular proliferation nuclear antigen (PCNA) and prevent the activation of DNA delta polymerase, which plays a role in DNA replication and repairing.

The p21 gene. At the transcriptional level, the p21 gene is the primary mediator in p53 – induced cell-cycle arrest although p21 can be induced on independent p53 pathways, as well. The cells without functional p53 (p53+) express only low levels of p21. Patients with p53-/p21+ have a significantly better prognosis in terms of recurrence, disease-free and overall survival and are much more sensitive to radiochemotherapy than the subgroup p53+/p21- (23,48). The stopping of the cellular cycle induced by radiation or apoptosis can be cancelled by the lack of p21 expression (23).

The p21 induction represents a new mechanism of resistance in rectal cancers treated with preoperative radiochemotherapy (49). In low and medium rectal cancers preoperatively treated with radiochemotherapy the p21 level correlated with the degree of tumoral invasion and survival (50). Along with stage and sex, p21 independently correlated with distal metastases in a group of patients treated exclusively by surgery (51).

The p16 gene is a suppressor gene inhibiting the CDK4/6 cycline dependent kinase thus preventing cell proliferation. In many human tumours the cellular cycle progression from phase G1 to phase S can be achieved by inactivating the retinoblastoma gene Rb, by inactivating the tumour suppressor protein p16 or by overexpression of D1 or CDK4 cycline. The loss of suppressor protein p16 expression can occur by the homozygotic gene deletion or by gene p16 methylation.

Within CRC, Palmquist defined two histological subtypes: 1 – small clusters with 2-5 cells/group situated at the invasion border; 2 – larger clusters making up most of the tumour mass. In the small clusters but not in the larger ones, p16 increases and the proliferation markers decrease (e.g., Ki-67). The nodal metastases occurring in CRC could originate from a special subclone of tumour cells, which had had an overexpression of p16 and low proliferative activity, i.e., which would originate from the small clusters of the invasion border. (22). In CRC, the decrease in p16 expression suggests a more aggressive potential of lymphatic infiltration and a less favourable prognosis. P16 over expression can also be correlated with stage N and mucinous and poorly differentiated histology (52).

The p27 gene. P27 was encountered in 44% of the 160 cases of rectal cancer; its presence was associated with local recurrence (35). The decrease in protein p27 expression can be associated with tumour size, positive lymph nodes and a lower survival rate (35,53). The higher p27 expression and lack of p53 is associated with a better prognosis (35). Some studies support the relevance of p27 for the metastatic disease. Although the cycline-D playing a role in proliferation can annihilate the p27 cellular cycle inhibitor, no correlation of p27, p53 or cycline-D1 with the lymph node status could be established.

In rectal cancer the presence of p27 does not predict the response to preoperative RCT (54). In the case of patients undergoing surgery for rectal cancer stage T2-3, the decrease in p27 expression could be correlated with a tendency of reducing the disease-free and overall survival (55).

E. Adhesion molecules

It is believed that tumour genesis and progression in CRC are influenced by the genetic accumulation of abnormalities in numerous suppressor and oncogenic genes including the nuclear overexpression of beta-catenine, p16 and c-myc as well as the loss of expression for E-cadherins.

Beta-catenine

In well-differentiated CRC there are genetic alterations at the level of the APC gene (mutated in 60-80% of the cases) or at the level of the oncogene beta-catenine (mutated in 12-48% of the cases), which stimulates the Wnt/wg pathway. Beta-catenine does not run down and it is accumulated in large concentrations in the tumour cells. Beta-catenine has the following functions:

- it is part of cell jonctions along with E-cadherins and it helps with maintaining intraepithelial adhesion thus playing a role in the progression of metastatic colorectal carcinomas (56);
- it is a transcription factor when it is situated in the nucleus (together with a member of the TCF/LEF – T cell factor/ lymphoid enhancer factor).

The in vitro experimental data indicate that beta-catenine favours the transcription of the c-myc gene and of cycline-

D1. In the center of the tumour the expression of beta-catenine and cycline-D1 is low whereas Ki-67 is increased (57). In colorectal carcinomas in which there are lymph node metastases and the serous membrane is affected there is also nuclear overexpression of beta-catenine and c-myc at the level of the tumour invasion area (58). Although it is involved in the initial phases of carcinogenesis in rectal cancer, the prognostic role of beta-catenine has not been proven by clinical studies yet.

F. Surface molecules

Muc-1. One of the characteristic features of glandular epithelial tissue is the synthesis and secretion of mucus – which consists of glycoproteins protecting the epithelial surfaces. In some malignant diseases including CRC mucin alterations by changing glycosylation and the production rate were reported. MUC-1 is a mucin antigen correlated with lymph node and liver metastases. Some studies suggest that an increase in the expression of the core peptide MUC-1 is associated with a poor prognosis. MUC-1 and MUC-2 are useful markers in defining the mucinous subtype of CRC. MUC-1 plays an important prognostic role in CRC in Caucasian patients but not in Afro-American ones; its prognostic value is not influenced by the tumour location (35).

2. DNA content in tumour cells

It is an important prognostic factor for some tumours. The cells containing a normal quantity of DNA are called diploid whereas those containing an abnormal quantity of DNA are aneuploid. A tumour may contain a single cell population (diploid or aneuploid) or two populations, a diploid one and an aneuploid one or two aneuploid ones in which case the tumour is called multiploid. The DNA index is used in the quantitative expression of the tumour DNA content. This index represents the ratio between the quantity of DNA in the tumour and the quantity of DNA in normal mucosa samples taken distally from the tumour location. For diploid tumours the DNA index is below 1.09 whereas for aneuploid tumours it is over 1.09.

In CRC, aneuploidy (detected by means of flow cytometry) is associated with a lower survival rate (59); this fact was not confirmed by all studies. Thus, in 653 patients operated on for CRC, aneuploidy was associated with a distal location of the tumour, perineural invasion, desmoplastic reaction and incapacity of secreting mucin but there was no correlation with survival (60).

In rectal cancer, aneuploidy and a proliferative index >10% were associated with a lower survival rate only in the univariate analysis; in the multivariate analysis only the tumour stage and the proliferative index maintained their prognostic value (61). However, other studies support the negative prognostic value of aneuploidy upon the survival rate .

In the case of aneuploid tumours the determination of the cell fraction in phase S of the cellular cycle before radiotherapy predicts a more frequent rate of recurrence (62). At the same time, the decrease in tumour diploidy and the preoperative proportion of postradiation necrosis are independent prognostic factors (63). Postoperative RCT in aneuploid tumours is associated with a less favourable prognosis (64).

3. Angiogenesis – recently, it has been regarded with more and more interest as a prognostic factor in certain solid tumours. The serum angiogenesis factors (serum vascular endothelial growth factor - VEGF and nitric oxide-NO) can be considered prognostic factors for disease-free survival in operable colorectal cancer (53,65). VEGF correlates with the p53 expression and phosphorylation of the Mitogen activated protein kinase p44 in CRC tumour cells (66).

In a group of 111 patients with colorectal cancer the tumoral microvascular density was evaluated; it was higher in the patients with a less favourable prognosis. Markers of certain histological tumour structures can be identified in the serum: TGF beta 1 (Transport Glucose Factor) expressed by cancerous cells, TGF beta 3 expressed by stromal cells and CD105 expressed by the endothelial cells of tumoral blood vessels. These markers may evaluate the progression of the disease (67).

The TGF beta 1 intratumorally – an expression of tumour hypoxia – is associated with a reserved prognosis of rectal tumours (68). Administration of the specific VEGF antibody to patients with rectal cancer reduces the vascular volume, the intratumoral perfusion and the microvessel density thus producing a rapid and direct antivascular effect in rectal tumours (69). Angiogenesis and the density of dendritic cells do not correlate with the presence of distal metastases in rectal cancer (70).

4. Neuroendocrine markers (chromogranin A, Syntaxin, synaptophysin) can be found in undifferentiated colonic and rectal tumours and are associated with a more aggressive development of the disease (71). In rectal adenocarcinoma the frequency and density of cells with an endocrine phenotype (chromogranin-positive) are higher in the patients who were given neoadjuvant therapy; the proportion of these cells correlates with the degree of response to treatment. The intensely chromogranin-positive cells have a lower proliferative index (expressed by Ki-67) but the p53 expression is higher. The mechanism of endocrine cells occurring in rectal cancers treated with radiochemotherapy is probably represented by the cytotoxic injury at the tumour level (72).

5. Proliferative activity – Ki-67 does not influence survival and disease-free survival.

6. Metalloproteinase inhibitors (TIMP1) correlate with survival in rectal cancer and are considered prognostic factors (73).

Topoizomerase I does not correlate with tumour stage, metastatic phenotype, presence of p53 or proliferative activity. Its expression should be evaluated in those patients who do not undergo treatment with topo-1 inhibitors in order to define better the role of this protein as a predictive marker (74).

Category IV: - includes well studied factors having no predictive value: macroscopic characteristics and tumour size.

Conclusion

In the future, attempts will be made to develop models of colorectal cancer in which age, along with other demographic characteristics, as well as the clinical, pathological and molecular stage will accurately predict the clinical evolution of patients and will help select specific therapies for different subgroups of patients with rectal cancer.

We expect the molecular prognostic factors to help identify – by preoperative biopsy – the patients with tumours sensitive to adjuvant therapy so that this therapy can be used selectively before surgery. However, robust statistical studies are still necessary in order to evaluate simultaneously - by multivariate analysis – as many markers as possible (and their interactions) before they can become the basis for therapeutic decisions or prognostic estimations.

References

1. Compton CC,Fielding LP, Burgart LJ, et al. Prognostic factors in colorectal cancer: College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med 2000; 124: 979-994.
2. Jessup JM, Stewart AK, Menck HR. The National Cancer Data Base report on patterns of care for adenocarcinoma of the rectum, 1985-95. Cancer 1998; 83:2408-2418.
3. Bilchik AJ, Nora DT, Sobin LH, et al. Effect of lymphatic mapping on the new tumor-node-metastasis classification for colorectal cancer. J Clin Oncol 2003; 21:668-672.
4. Fisher ER, Colangelo L,Wieand S, Fisher B, Wolark N. Lack of influence of cytokeratin-positive mini micrometastases in " Negative Node " patients with colorectal cancer: findings from the national surgical adjuvant breast and bowel projects protocols R-01 and C-01. Dis Colon Rectum 2003, 46:1021-1026
5. Wiggers T, Arends JW, Volovics A. Regression analysis of prognostic factors in colorectal cancer after curative resections. Dis Colon Rectum 1988; 31: 33-41.
6. Compton C, Fenoglio-Preiser CM, Pettigrew N, Fielding LP. American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Working Group. Cancer 2000; 88:1739-1757.
7. Quirke P, Durdey P, Dixon MF, Williams NC. Local reccurence of rectal adenocarcinoma due to inadequate surgical resection. Histopathological study of lateral tumour spread and surgical excision. Lancet 1986; 2 (8514):996-999.
8. Tomita N, Monden M, Kikkawa N et al. Analysis of microsatellite instability and p53 LOH in advanced colorectal cancers-first report of the No. 3 protocol. Gan To Kagaku Ryoho.2004; 31:367-372.
9. Hoogerbrugge N, Willems R, Van Krieken HJ et al. Very low incidence of microsatellite instability in rectal cancers from families at risk for HNPCC. Clin Genet 2003; 63:64-70.
10. Iino H, Fukayama M,Maeda Y et al. Molecular genetics for clinical management of colorectal carcinoma 17p, 18q, and 22q loss of heterozygozity and decreased DCC expression are correlated with the metastatic potential. Cancer 1994; 73: 1324-1331.
11. Gotley DC, Reeder JA, Fawcett J et al. The deleted in colon cancer (DCC) gene is consistently expressed in colorectal cancers and metastases.Oncogene 1996; 13:787-795.
12. Cohn KH, Ornstein DL, Wang F et al. The significance of allelic deletions and aneuploidy in colorectal cancer. Results of a 5-year follow-up study. Cancer 1997; 79:233-244.
13. Shibata D, Reale MA, Lavin P et al. The DCC protein and prognosis in colorectal cancer. N Engl J Med 1996; 335:1727-1732.
14. Saito M, Yamaguchi A, Goi T et al. Expression of DCC protein in colorectal tumors and its relationship to tumor progression and metastasis. Oncology 1999; 56: 134-141.
15. Reymond MA, Dworak O, Remke S et al. DCC protein as a predictor of distant metastases after curative surgery for rectal cancer. Dis Colon Rectum 1998; 41:755-760.
16. Morgan M, Koorey D, Painter D et al. P53 and DCC immunohistochemistry in curative rectal cancer surgery. Int J Colorectal Dis 2003;18:188-195.
17. Ruo L, Tickoo S, Klimstra DS et al. Long- term prognostic significance of extent of rectal cancer response to preoperative radiation and chemotherapy. Ann Surg 2002, 236 (1):75-81.
18. Ueno H, Hase K, Mochizuki H. Criteria for extramural peri-neural invasion as a prognostic factor in rectal cancer. Br J Surg 2001, 88(7): 994-1000.
19. Okuyama T, Oya M, Ishikawa H. Budding as a useful prognostic marker in pT3 well- or moderately- differen-tiated rectal adenocarcinoma. J Surg Oncol 2003; 83: 42-47.
20. Masaki T, Matsuoka H, Sugiyama M et al. Budding as a useful determinant of the optimal treatment for T1 rectal carcinomas. Hepatogastroenterology 2003; 50:388- 391.
21. Ueno H, Jones AM, Wilkinson KH et al. Histological categorisation of fibrotic cancer stroma in advanced rectal cancer. Gut 2004; 53: 581-586.
22. McKay JA, Douglas JJ, Ross VG et al . Analysis of key cell-cycle checkpoint proteins in colorectal tumors. J Pathol 2002; 196:386-393.
23. Schwandner O, Bruch HP, Broll R. p21, p27 , cyclin D1, and p53 in rectal cancer: immunohistology with prognostic significance? Int J Colorectal Dis 2002; 17:11-19.
24. Rebischung C, Gerard JP, Gayet J et al. Prognostic value of p53 mutations in rectal carcinoma.Int J Cancer 2002; 100: 131-135.
25. Klump B, Nehls O, Okech T et al. Molecular lesions in colorectal cancer: impact on prognosis? Original data and review of the literature. Int J Colorectal Dis 2004 ; 19: 23-42.
26. Subramaniam P, Sinha SN, Wilkinson S. Immunohistochemical detection of p53 and the recurrence of rectal adenocarcinoma. Aust NZJ Surg 1995; 65:740-742.
27. Liang JT, Cheng YM, Chang KJ et al. Reappraisal of K-ras and p53 mutations in recurrence of Duke's B2 rectal cancer after curative resection. Hepatogastroenterology 1999; 46: 830-837.
28. Adell G, Sun XF, Stal O et al. P53 status: an indicator for the effect of preoperative radiotherapy of rectal cancer. Radiother Oncol 1999; 51:169-174.

29. Luna-Perez P, Arriola EL, Cuadra Y et al. P53 protein overexpression and response to induction chemoradiation therapy in patients with locally advanced rectal adenocarcinoma. Ann Surg Oncol 1998; 5: 203-208.
30. Elsaleh H, Robbins P, Joseph D et al. Can p53 alterations be used to predict tumour response to pre-operative chemo-radiotherapy in locally advanced rectal cancer? Radiother Oncol 2000; 56:239-244.
31.Wiggenraad R, Tamminga R, Blok P et al. The prognostic significance of p53 expression for survival and local control in rectal carcinoma treated with surgery and postoperative radiotherapy. Int J Radiat Oncol Biol Phys 1998; 41: 29-35.
32. Sato T, Nishimura G, Fushida S et al. Evaluation of p53, Ki-67 and DNA ploidy in both primary rectal carcinomas and locally recurrent tumors. Oncol Rep 1998; 5: 1225-1229.
33. Dix BR, Robbins P, Soong R et al. The common molecular genetic alterations in Dukes B and C colorectal carcinomas are not short term prognostic indicators of survival. Int J Cancer 1994; 59: 747-751.
34. Elsaleh H, Soontrapornchai P, Grieu F at al. P53 alterations have no prognostic or predictive significance in Dukes' C rectal carcinomas. Int J Oncol 1999; 15: 1239-1243.
35. Grizzle WE, Manne U, Weiss HL et al. Molecular staging of colorectal cancer in African-American and Caucasian patients using phenotypic expression of p53, bcl-2, MUC-1 and p-27 (kip-1).Int J Cancer 2002; 97: 403-409.
36. Kapiteijn E, Liefers GJ, Los LC et al. Mechanisms of oncogenesis in colon versus rectal cancer. J Pathol 2001; 195: 171-178.
37. Rebischung C, Laurent-Puig P, Gerard JP et al. Analysis of genetic disorders of cancer of the rectum: differences in relation to cancer of the colon. Gastroenterol Clin Biol 1998; 22: 679-687.
38. Ishijima N, Miki C,Ishida T et al. The immunohistochemical expression of BCL-2 oncoprotein in colorectal adenocarcinoma. Surg Today 1999; 29: 682-684.
39. Bhatavdekar JM, Patel DD, Ghosh N et al. Coexpression of Bcl-2, c-Myc, and p-53 oncoproteins as prognostic discriminants in patients with colorectal carcinoma. Dis Colon Rectum 1997; 40: 785-790.
40. Schwandner O, Schiedeck TH, Bruch HP et al. P53 and Bcl-2 as significant predictors of reccurence and survival in rectal cancer. Eur J Cancer 2000; 36: 348-356.
41. Rodel F, Hoffmann J, Grabenbauer GC et al. High survivin expression is associated with reduced apoptosis in rectal cancer and may predict disease-free survival after preoperative radiochemotherapy and surgical resection. Strahlenther Onkol 2002; 178: 426- 435.
42. Andreyev HJ, Norman AR, Cunningham D et al. Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 1998; 90: 675-684.
43. Frattini M, Balestra D, Suardi S et al. Different genetic features associated with colon and rectal carcinogenesis. Clin Cancer Res. 2004 ; 10:4015-4021.
44. Luna-Perez P, Segura J, Alvarado I et al. Specific c-K-ras gene mutations as a tumor response marker in locally advanced rectal cancer treated with preoperative chemo-radiotherapy. Ann Surg Oncol 2000; 7: 727-731.
45. Kornmann M, Link KH, Galuba I et al. Association of time to recurrence with thymidylate synthase and dihydropyrimidine dehydrogenase mRNA expression in stage II and III colorectal cancer. J Gastrointest Surg 2002; 6: 331-337.
46. Saw RP, Morgan M, Koorey D et al. P53, deleted in colorectal cancer gene, and thymidylate synthase as predictors of histopathologic response and survival in low, locally advanced rectal cancer treated with preoperative adjuvant therapy. Dis Colon Rectum 2003; 46:192-202.
47. Okonkwo A, Musunuri S, Talamonti M et al. Molecular markers and prediction of response to chemoradiation in rectal cancer. Oncol Rep.2001; 8: 497-500.
48. Bukholm IK, Nesland JM. Protein expression of p53 , p21 (WAF 1/CIP1), bcl-2, Bax , cyclin D1 and pRb in human colon carcinomas. Virchows Arch 2000; 436:224-228.
49. Rau B, Sturm I, Lage H et al. Dynamic expression profile of p21 WAF1/CIP 1 and Ki67 predicts survival in rectal carcinoma treated with preoperative radiochemotherapy. J Clin Oncol 2003; 21(18): 3391-3401.
50. Sogawa N, Takiguchi N, Koda K et al. Value of expression of p21 WAF1/CIP1 as a prognostic factor in advanced middle and lower rectal cancer patients treated with preoperative radio-chemotherapy. Int J Oncol 2002; 21:787-793.
51. Schwandner O, Bruch HP, Broll R. Prognostic significance of p21 and p27 protein , apoptosis, clinical and histologic factors in rectal cancer without lymph node metastases. Eur Surg Res 2002; 34(6): 389-396.
52. Tada T, Watanabe T, Kazama S et al. Reduced p16 expression correlates with lymphatic invasion in colorectal cancers. Hepatogastroenterology 2003; 50:1756-60.
53. Akbulut H, Altuntas F, Akbulut KG et al. Prognostic role of serum vascular endothelial growth factor , basic fibroblast growth factor and nitric oxide in patients with colorectal carcinoma. Cytokine 2002; 20 (4):184-190.
54. Gunther K, Dimmler A, Rodel F et al. P 27 does not predict histopathological response to radiochemotherapy in rectal cancer. J Surg Res 2003; 113: 179-188.
55. Hoos A, Nissan A, Stojadinovic A et al. Tissue microarray molecular profiling of early, node-negative adenocarcinoma of the rectum: a comprehensive analysis. Clin Cancer Res 2002; 8 (12):3841-3849.
56. Ikeguchi M, Makino M, Kaibara N. Clinical significance of E-cadherin-catenin complex expression in metastatic foci of colorectal carcinoma. J Surg Oncol 2001; 77: 201-207.
57. Jung A, Schrauder M, Oswald U et al. The invasion front of human colorectal adenocarcinomas shows co-localisation of nuclear beta-catenin, cyclin D1 and p16 INK 4A and is a region of low proliferation. Am J Pathol 2001; 159:1613-1617.
58. Xie D, Sham JS, Zeng WF et al . Heterogenous expression and association of betacatenin, p16 and c-myc in multistage colorectal tumorigenesis and progression detected by tissue microarray. Int J Cancer 2003, 107: 896-902.
59. Kokal W, Sheibani K, Terz J et al. Tumor DNA content in the prognosis of colorectal carcinoma. JAMA 1986; 255: 3123-3127.
60. Tang R, Ho YS, You YT et al. Prognostic evaluation of DNA flow cytometric and histopathologic parameters of colorectal cancer. Cancer 1995; 76:1724-1730.
61. Berczi C, Bocsi J, Bartha I et al. Prognostic value of DNA ploidy status in patients with rectal cancer. Anticancer Res 2002; 22 : 3737-3741.
62. Lammering G, Taher MM, Borchard F et al. The prognostic value of S-phase fraction in preoperative radiotherapy of rectal cancer. Oncol Rep 2001 Jan-Feb; 8(1): 201-206.

63. Lammering G, Taher MM, Gruenagel HH et al. Alteration of DNA ploidy status and cell proliferation induced by preoperative radiotherapy is a prognostic factor in rectal cancer. Clin Cancer Res 2000; 6: 3215-3221.
64. Tang R, Ho YS, Chen HH et al. Different prognostic effect of postoperative chemoradiation therapy on diploid and nondiploid high-risk rectal cancers. Dis Colon Rectum 1998; 4: 1494-1499.
65. Nakayama Y, Sako T, Shibao K et al. Prognostic value of plasma vascular endothelial growth factor in patients with colorectal cancer. Anticancer Res 2002; 22:2437-2442.
66. Cassano A, Bagala C, Battelli C et al. Expression of vascular endothelial growth factor, mitogen-activated protein kinase and p53 in human colorectal cancer. Anticancer Res.2002;22:2179-84.
67. Li C, Gardy R, Seon BK et al. Both high intratumoral microvessel density determined using CD105 antibody and elevated plasma levels of CD105 in colorectal cancer patients correlate with poor prognostic. Br J Cancer 2003; 88: 1424-1431.
68. Cooper R, Sarioglu S, Sokmen S et al. Glucose transporter-1 (GLUT-1) : a potential marker of prognosis in rectal carcinoma? Br J Cancer 2003; 89: 870-876.
69. Willett CG, Boucher Y, di Tomaso E et al. Direct evidence that the VEGF specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 2004; 10:145-147.
70. Gunther K, Radkow T, Reymond MA et al. Angiogenesis and dendritic cell density are not correlated with metachronous distant metastasis in curatively operated rectal cancer. Int J Colorectal Dis 2003; 18: 300-308.
71. Grabowski P , Schonfelder J, Ahnert-Hilger G et al. Expression of neuroendocrine markers: a signature of human undifferentiated carcinoma of the colon and rectum. Virchows Arch 2002; 441:256-263.
72. Shia J, Tickoo SK, Guillem JG et al. Increased endocrine cells in treated rectal adenocarcinomas: a possible reflection of endocrine differentiation in tumor cells induced by chemotherapy and radiotherapy. Am J Surg Pathol 200;26:863-872.
73. Holten- Andersen M, Christensen IJ, Nilbert M et al. Association between preoperative plasma levels of tissue inhibitor of metalloproteinases 1 and rectal cancer patient survival, a validation study. Eur J Cancer 2004; 40: 64-72.
74. Boonsong A, Curran S, McKay JA et al. Topoisomerase I protein expression in primary colorectal cancer and lymph node metastases. Hum Pathol 2002;33:1114-1119.