THE MOLECULAR CHARACTERISTICS OF THE TUMOUR AND THE PROGNOSIS OF COLORECTAL CARCINOMA

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THE MOLECULAR CHARACTERISTICS OF THE TUMOUR AND THE PROGNOSIS OF COLORECTAL CARCINOMA (ABSTRACT): Due to the high rate of incidence and the overall mortality, colorectal cancer is one of the most important medical problems modern medicine has to deal with. A lot of research has been done in order to understand the molecular basis of the carcinogenesis process in colorectal adenocarcinoma and now the evolution of the colorectal cancer from normal epithelial cell to cancer cell is one of the best understood models of carcinogenesis. In order to fully understand this process one must have basic knowledge of the histology and the genetics of colorectal adenocarcinoma. Also of great importance is the role that the immune system has to play in the development and progression or regression of this type of cancer – from the basic immune response to the presence of the neoplastic cells, to the immune response that is caused by central necrosis which can lead to stimulation of tumour growth and finally to the immune response that is responsible for the development of cancer in the presence of chronic inflammation. The reason why the biology of the colorectal cancer is so important is that it can not only lead to finding new ways of treatment but mostly because it can lead to a better staging system than the classical Dukes staging system, which in turn can correct under-treatment or over-treatment of colorectal cancer lesions.

KEY WORDS: COLORECTAL CANCER, MUTATIONS, SPECIFIC IMMUNE RESPONSE, INNATE IMMUNE RESPONSE, IMMUNE SURVEILLANCE

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INTRODUCTION

Colorectal cancer is one of the most well known cancers mainly in terms of the genetic alterations which lead from a normal epithelial cell to a cancer cell. Despite these recent advances, the Duke's staging system (modified by Astler and Coller in 1954) and the TNM staging - based solely on the histological characteristics of the tumour - are the only tools widely used in assessing the prognosis of a colorectal neoplastic lesion and this has major implications in the therapeutic decisions made for each patient.

A "top-down" strategy for gathering and integrating information about various other factors that could influence the prognosis of the tumour should include the assessment of the histological type of the tumour, followed by the analysis of the tumour microenvironment and finally the molecular alterations responsible for the development of the tumour.

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Such an analysis could lead to a better understanding of the factors that play a role in determining a tumour's outcome and it could complete the prognosis issued by the TNM staging thus leading to a better patient management.

COLON CANCER HISTOLOGY

Macroscopy

The macroscopic aspect of colorectal cancer lesions varies from exofytic lesions that develop into the lumen (which are moreover seen in the right colon) to ulcerated, infiltrative lesions which develop into the wall of the colon and tend to become circumferential (they are mostly seen in neoplastic lesions of the left colon – Fig. 1). Most of the tumours are however a mixture of these macroscopic types [1].

Microscopy

According to the World Health Organization's (WHO) recommendations in the case of colonic neoplasia the term colon cancer is only used in those cases in which the neoplasic proliferation invades the *submucosa* eroding *muscularis mucosae*. Therefore lesions that have characteristics similar to those seen in colon cancer but are limited to the mucosa should not be termed "adenocarcinoma in situ" but high grade intra-epithelial neoplasia [2]. The main reason behind these recommendations is the difference in the prognosis of those lesions compared to cancer.

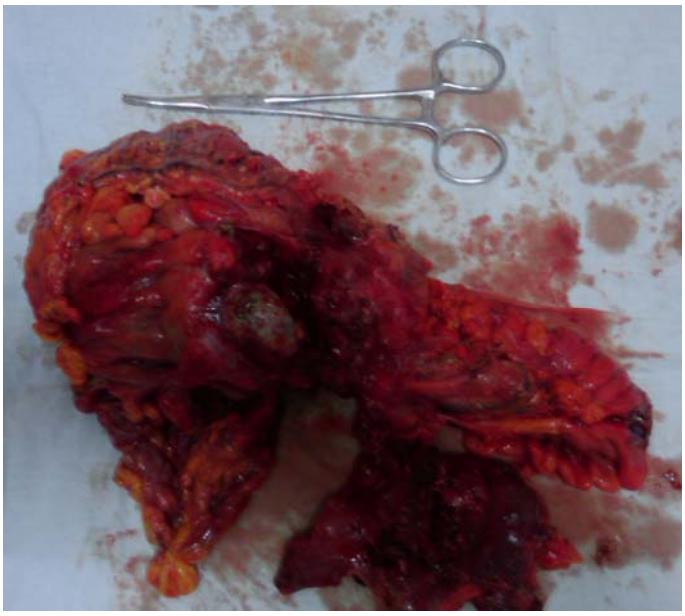

Fig.1 *Macroscopy of an extremely aggressive tumour located in the descending colon of a 27 year old patient with significant familial aggregation of colon cancer. The tumour was invading the left ureter.*
(image from Prof. St. O. Georgescu collection)

Cells that belong to adenocarcinomatous lesions of the colon are tall, mucus secreting cells that can form glandular structures containing mucus and cellular debris, depending on the degree of differentiation of the cells.

The following types of adenocarcinomas can be described depending on the morphology of the cells that constitute them:

- mucinous – more than 50 % of the lesion is represented by mucine;
- "signet ring" cell carcinoma – more than 50 % of the cells that constitute the tumor show large intracellular quantities of mucine that displace the nucleus giving the cell its characteristic aspect;
- adenosquamous carcinoma – the tumour is comprised of both squamous and mucinous cells;
- medullary carcinoma – prominent nucleoli and pink abundant cytoplasm;
- undifferentiated carcinoma – the cells barely resemble epithelial cell from which they originate;
- carcinosarcoma – cells that constitute this type of tumour are positive for cytokeratine and show both epithelial and mesenchymal origin.

Cell differentiation

The WHO recognizes the classification of colon cancer tumours into well differentiated, moderately differentiated and poorly differentiated based on the degree of cellular differentiation. Another classification could be into high-grade differentiation and low-grade differentiation.

If within a tumour there are areas with different degrees of differentiation the degree of differentiation of the tumour will be that of the least differentiated area (not taking into account the invasion front of the tumour).

Progression stages from normal mucosa to adenocarcinoma

The first morphological alterations on the progression from a normal epithelial cell to a malignant lesion are the aberrant crypt foci – areas of thickened epithelium in the mucosa which show enlarged crypts. From microscopic point of view these areas resemble hyperplasic polyps (which generally associate a high incidence of *ras* gene mutations) or dysplastic polyps (which involve mostly APC gene mutations).

The following stage is adenoma – characterized by hyperchromatic, atypical nuclei and hypercellularity (intraepithelial neoplasia). These lesions are associated at a molecular level with the inactivation of the *Wnt* – pathway and the anatomical progression of these lesions takes place from the crypts upwards to the luminal surface [3].

There have been reported cases of adenocarcinomas that do not follow the adenoma-adenocarcinoma sequence and there are adenocarcinomas that develop based on other precursor lesions as is the case of Peutz-Jeghers polyps or that of the hamartomatous polyps.

Molecular biology of colorectal cancer

Adenocarcinomas of the colon can be divided into two groups based on the type of genetic alterations that induce cancer appearance. The first group is characterized by allelic loss which can affect the short arm of chromosome 17 or the long arm of chromosome 18. Due to the aneuploidy that results this group is termed LOH + (Loss Of Heterozygosis)

In the second group the genetic alterations are due to microsatellite instability – the karyotype of these tumours is normal but mutations occur due to errors in DNA replication and therefore this group is called RER+ (Replication Error Repair).

APC gene and p53 gene mutations are characteristic to the first group while *ras* gene mutations mainly appear in RER+ tumors [4].

LOH+ cancers constitute almost 2/3 of all colorectal adenocarcinomas and these types of genetic alterations are more frequently found in the tumours of the left colon. The triggering event that leads to carcinogenesis seems to be a mutation affecting the *Wnt* pathway – a complex network of proteins involved in embryogenesis and intercellular adhesion (Fig. 2). In normal cells the coupling of the *Wnt* protein to its receptor starts a pathway of molecular events leading to the entrance of β-catenine into the nucleus and the activation of the transcription of a gene that stimulates cell proliferation [3].

The APC gene encodes a protein forming a complex with β-catenine and GSK-3β (glycogen syntase-kinase 3β). GSK-3β is responsible for the inactivation of β-catenine, but the complex can only be formed in the presence of the APC protein. β-catenine activity can also be modulated by the cycline D1, gastrine or PPAR-β (peroxysome proliferator activated receptor β).

Due to the role it has as a control factor of cellular proliferation induced by β-catenine the APC gene is considered to be a tumour-suppressor gene which requires a mutation of both its alleles in order to trigger a carcinogenetic effect [3].

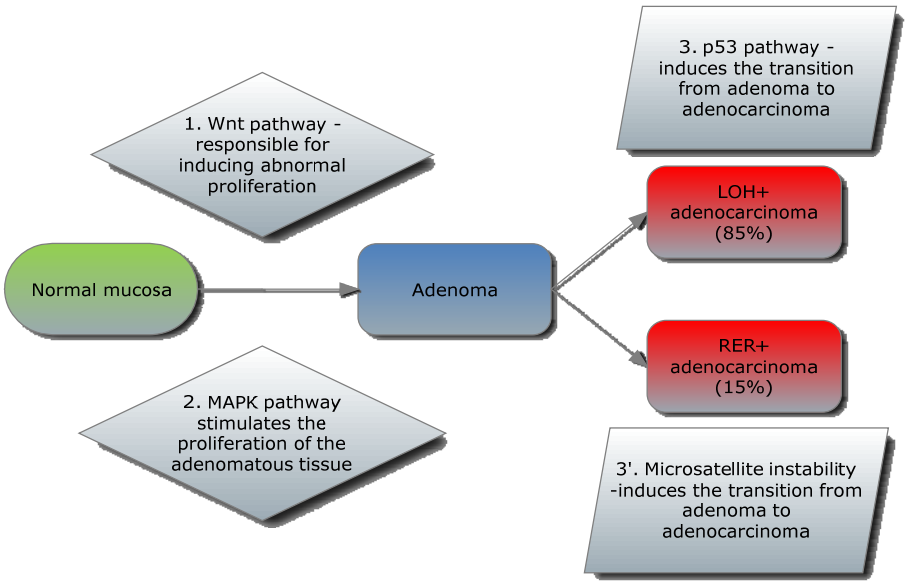

Fig. 2 *Genetic alterations in colorectal carcinogenesis*

Some of the latest data in the literature suggest that the interaction of the APC protein with β-catenine blocks any interaction between β-catenine and E-cadherine, thus we may conclude that APC gene mutations also lead to a loss of the normal contact inhibition which is responsible for limiting excessive cellular proliferation.

The mutations of the *ras* genes, the p53 gene or the DCC (deleted in colon cancer) gene are also involved in colorectal carcinogenesis. The mutation of the *K-ras* gene is alleged to facilitate the growth of the adenomatous tissue, while mutations of the p53 gene and those of the DCC gene play a part in the transition from adenoma to adenocarcinoma.

The p53 gene mutations are the second most frequent mutation after the APC gene mutations and just like the APC gene it is also a tumour suppressor gene. This gene is responsible for blocking the cell cycle in order to allow DNA repair or if repair is impossible it induces apoptosis.

Hamelin et al. have shown that p53 gene mutations are themselves a predictive factor in assessing the prognosis of colorectal cancer patients, independently from any pathology based prognosis. The study that took place under Hamelin's supervision enrolled 192 patients and proved not only that p53 gene mutation can be an independent prognosis factor but also that different mutations have different prognostic value [6].

Different articles in the literature show different results when it comes to investigating the role that the p53 gene mutations play and the main reason for that is the use of different methods to evaluate these mutations – direct methods that study the gene itself vs. indirect methods that investigate the gene product (Table 1). As expected the best way to evaluate p53 gene mutations is the direct way by molecular studies of the gene itself [7,8].

Table 1

Studies assessing the impact of p53 gene mutations on the prognosis of different cancers – adapted from Petitjean et al – Impact of mutant p53 functional properties on TP53 mutation patterns and tumor phenotype: lessons from recent developments in the IARC TP53 database [8]

Tumor location	Number of studies on p53 gene mutations		
	associated with bad prognosis	associated with good prognosis	irrelevant on the prognosis
colorectal	16	0	8
brain	3	2	4
esophagus	2	0	2
liver	3	0	0
larynx	0	0	1
bone	1	0	1
ovary	7	1	2
pancreas	1	1	1
lung	8	0	6
prostate	1	0	1
breast	28	1	5
stomach	1	0	2
urinary bladder	4	0	3

The DCC gene is a tumour suppressor gene that has a role in inducing cell diferentiation in normal mucosa. These mutations are present in 70 % of all colorectal cancers, but it is yet uncertain if the mutations are a trigger for carcinogenesis or if the mutations are themselves caused by other mutations [2].

The product of the *K-ras* gene is a G protein that is involved in the transduction of the signal through the cellular membrane obtaining energy for this process via the transformation of GTP to GDP. Its mutations cause a defective G protein that is incapable of performing GTP hydrolysis and therefore remains permanently active causing uncontrolled cell proliferation [1].

The mutations of genes from the ras family are typical for the RER + colorectal cancers. Genetic studies of patients with cancers that belong to this group show normal karyotype as opposed to LOH+ cancers and this leads to the conclusion that these mutations appear in single cells as errors in DNA replication. This type of mutations often has a better clinical outcome than LOH+ tumors and is associated with the mucinous adenocarcinomas.

Medullary adenocarcinoma is also linked to microsatellite instability as opposed to „signet ring" cell carcinomas which are rarely associated with DNA replication errors and are more aggressive.

These associations make obvious the role that the type of mutation involved in carcinogenesis plays in determining the outcome of the tumour [6].

The immune system and colorectal cancer

Rudolf Virchow was the first to make a connection between the immune response and cancer. Almost a hundred years ago he showed an abundant leucocytic infiltrate in the tumours he was studying.

An immunologic approach to the study of carcinogenesis in general is based on the fact that cancer can be a result of chronic inflammation as is the case of lung cancer where cigarette smoke plays an iritative role that induces an inflammatory response [9].

Other examples can be oesophageal cancer developed on a long lasting oesophagitis, or melanoma that develops on skin that is exposed to UV radiation for long periods of time [10].

On the other hand inflammation can play a positive role in protecting against cancer, a role that can be exploited in cancer therapy [11]. It is not easy to say that the immune response plays a tumour suppressing role or a tumour promoting role but rather that the immune systems interacts with cancer cells through a complicated web-like network of responses (Fig. 3) [12].

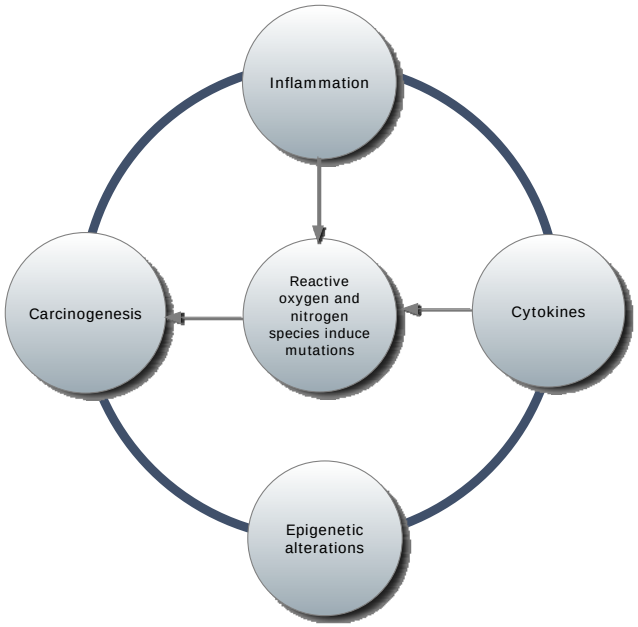

Fig. 3 *Role of inflammation in initiating and promoting carcinogenesis – adapted fromGrivennikov et al - Immunity, inflammation, and cancer [4].*

Types of inflammation in cancer

Chronic inflammation such as the one seen in Helicobacter pylori, or hepatitis B and C infections generally leads to carcinogenesis whereas acute inflammation such as different infections of the urinary tract have a beneficial effect on bladder cancer [13]. The intimate details of the reasons why chronic inflammation and acute inflammation seem to play different roles are not well understood and the problem can become even more complicated when one considers that chronic psoriatic inflammation can protect against carcinogenesis [14].

Mutations of ras genes or myc genes tend to induce not only an aberrant cell proliferation but also a remodelling process of the tumour microenvironment which leads to leukocyte recruiting and chemokine production that induces carcinogenesis and neo-angiogenesis.

Necrotic cells at the centre of the tumour where the blood supply is insufficient release inflammatory molecules such as IL-1 and HMGB-1 (high-mobility group protein B1) that stimulate neo-angiogenesis which allows peripheral proliferation of the tumour [2,15].

Another type of inflammation that is only present in some tumours is a result of active secretion of proinflammatory molecules which is a process similar to the one that induces wound healing – a reason why cancers are called „wounds that never heal" [1,16]. These type of tumours usually develop on a pre-existing lesion that necessitates an immune response that soon becomes aberrant and self-sustained [9].

A third type of immune response associated to cancer is the one seen after radiotherapy or chemotherapy. This inflammatory process can be explained via the same mechanisms as the ones seen in hypoxic necrosis [17].

Through the induction of neo-angiogenesis this process can support carcinogenesis but by enhancing epitope presentation it can also limit tumor growth [18]. This is the reason why anti-inflammatory drugs such as NSAIDs or corticosteroids can be used together with radiotherapy or chemotherapy to reduce the tumour promoting effect of the therapy [19].

Immune cells involved in carcinogenesis

Because of these various types of immune responses it is obvious that the mass of the tumour will contain immune cells besides tumour cell and the supporting stroma. These immune cells will form networks of intercellular signalling by direct contact or by signalling molecules. These signalling molecules are the ones that can shift the balance from a tumour promoting to a tumour suppressing immune response [20,21].

Innate immune response

The innate immune response plays a role in the recognising stressed cells and the products resulting from non-apoptotic or non-autophagic cell death. Solid tumours most often contain macrophages that appear to stimulate tumor growth and are major players in neo-angiogenesis, invasion and metastasis. This is why an abundant macrophage infiltrate is associated with a bad prognosis [22].

A high eosinophile blood count and an eosinophilic infiltrate in the tumor are linked to a good prognosis for the patient – eosinophilia is present after efficient chemotherapy, especially after treatments with IL-2 [23].

Acquired immune response

T-cells can be classified into 2 major groups based on the type of receptor: γδ and αβ.

The ones with αβ type receptors can further be divided into CD8+ - cytotoxic cells, CD4+ - helper cells and NK – natural killer cells. The CD4+ lymphocytes can in turn be subdivided into Th1, Th2, Th17 and T regulatory (Treg cells).

T-cells can play both a tumour promoting role and a tumor suppressing role. Cytotoxic T-cells and Th1 cells can recognize aberrant molecules that are a result of the mutagenteic process and thus are moreover associated with a good prognosis if they are found in large numbers in colorectal tumours [24 - 26]. Treg cells usually carry a less favourable prognosis [5].

Antigen presenting cells also play a very important role in facilitating the recognition of malignant cells from normal cells. Antigens derived from malignant cells can be processed and presented by dendritic cells to CD8+ cells despite the lack of MHC class I molecules on the tumour cells (the absence of MHC class I molecules is a direct consequence of the selection pressure that is imposed on the tumour by the immune surveillance of the immune system) [27].

One strategy of stimulating a tumour suppressing immune response could be stimulating epitope presentation through α or γ interferon or through cytokines like IL-2 that recruit immune cells [28,29]. Specific epitope presentation can be enhanced by the use of FLT3-ligand, GMCSF or IL-3 [29].

On the other hand an inflammatory microenvironment can induce the production of different mutations that promote carcinogenesis through the production of reactive oxygen or nitrogen species. Genomic and metabolic stress can be recognized by the immune system through the presence of molecules such as Rae-1, MICA and MICB or via the release of cytoscheletal components such as heparan sulphate [30]. These molecules are recognised by toll-like receptors, NOD-1 like receptors (that recognize uric acid) and RIG-I like receptors (they can recognize RNA fragments) [31].

Most of the molecules above are a result of cell death via apoptosis or necrosis and are part of a larger family of molecules entitled DAMP (disease associated molecular patterns) which includes purine metabolites like uric acid, ATP, hialuronan, HSPs, heparan sulphate and sindecan [32,33]. HMGB-1 is part of this family and is released after the death of the cell through necrosis and apoptosis and its release increases following radiotherapy [33,34].

As a conclusion we could say that the study of the immune response involved in cancer can answer with higher precision to the question of colorectal cancer prognosis compared to a simple histological evaluation.

There is already evidence in the literature that shows a link between the presence of Th1 lymphocytes and a more favourable evolution. On the other hand there is also evidence of a bad prognosis associated to a strong specific immune response even in small tumours with minimal local extension [4,35].

We should also ask 2 major questions that must be answered in the future regarding the microenvironment of colorectal cancer tumours and their prognosis:

1. can the assessment of the immune response bring more information on the possible evolution of the tumour, different from that already obtained from the TNM staging of the tumour?
2. what kind of inflammatory infiltrate is associated with a favourable prognosis and which is not?

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