2. Molecular biology of colorectal cancer

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Abstract. An estimated one million people in the world are diagnosed each year with CRC cancer. Over 30% of these people will die of disseminated disease. In recent years, we have furthered our knowledge of the molecular mechanisms responsible for the onset, development, and spread of the disease to other organs. The Vogelstein model of CRC tumorigenesis, published in the early 1990s, pioneered our understanding of how solid tumours developed. Major advances in the field of tumour neoangiogenesis and greater knowledge of the function of epidermal growth-factor receptors have since added new specifically targeted drugs, such as bevacizumab, cetuximab, and panitumumab, to the armamentarium. Moreover, we know that certain changes in the intracellular signalling cascade triggered by these receptors can influence drug response. In this chapter we summarise our knowledge of tumour-cell physiology itself, the microenvironment surrounding colon cancer, and the possible involvement of epigenetic phenomena and stem cells in the genesis and development of these tumours.

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Introduction

The relevance of CRC in neoplastic diseases and general gastrointestinal disorders has been amply demonstrated. With a worldwide incidence of more than one million cases a year, CRC is the second leading cause of cancer deaths in adults, with a mortality rate of 33% in developed countries [1]. CRC is a paramount health problem for the World Health Organization (WHO).

Despite advances in screening, diagnostics, and treatment in this field in recent decades, which have led to increased cure and response rates and improved quality of life, much of the natural history of the disease, especially its origins, remains unknown. Research in recent years has thus focused on the molecular biology of the disease. The underlying goal is not only to improve prevention and early diagnosis, but also to discover new weapons to fight the disease [2].

Advances in molecular biology have led to new therapeutic targets and new molecular prognostic markers and predictors of response to current treatments [3]. The aim is to try to individualise treatments, in accordance with the tumour's characteristics, and to obtain therapies that act directly on the tumour [4]. Our knowledge of how tumours develop, however, is still insufficient; we lack a complete understanding of carcinogenesis at the molecular level. We continue to investigate the different pathways by which a normal mucosa or a simple adenomatous polyp turns into a neoplasia, with the ability to infiltrate and trigger distant dissemination.

Understanding these molecular pathways will enable us to generate hypotheses for more-specific and less-toxic future therapies and molecular treatments that will also be much more effective. The great hope for the future appears to lie in increasing our knowledge of the molecular biology of CRC [5].

Sporadic versus hereditary molecular bases of colorectal cancer

About 20-30% of all colorectal cancers are due to a hereditary, or familial predisposition. Patients with a history of first or second degree relatives with CRC have a 20% higher risk of suffering the disease. This risk increases considerably, to 80-100% in some cases, if the disease is associated with specific hereditary syndromes [6].

Cases associated with well-defined hereditary syndromes like Lynch Syndrome or Familial Adenomatous Polyposis account for less than 5% of all colorectal cancers. The remaining 20-25%, associated with some type of hereditary form, tend to occur as a result of polymorphisms or low-
penetrance mutations with no clear association to a clear hereditary syndrome [6]. These cases are less genetically determined than with hereditary syndromes, which we will only briefly discuss, outlining just a few of their characteristics, as they are discussed in much greater depth elsewhere in this book.

**Hereditary nonpolyposis colorectal cancer (HNPCC), or Lynch syndrome**

HNPCC accounts for 2-4% of all colorectal cancers. Patients have an 80% risk of developing cancer during their lives. HNPCC is an autosomal dominant hereditary condition. It is associated with endometrial tumours (40-60%) and less commonly with gastric, ovarian, urinary, bowel, pancreatic, and brain tumours [7]. It is genetically characterised by high levels of microsatellite instability due to mutations in genes of the mismatch-repair (MMR) system. In 90% of cases, mutations are found in MSH2 or MLH1, and occasionally in MSH6 or PMS2 [8]. Apart from the diagnosis of HNPCC through genetic testing, clinical criteria can also be applied, as described in the Amsterdam II Criteria and Bethesda guidelines.

**Familial adenomatous polyposis (FAP)**

FAP accounts for about 1% of all colorectal cancers. It is characterised by the appearance of hundreds to thousands of adenomatous polyps in the colon during adolescence. It is an autosomal dominant hereditary disorder, with 100% penetrance (if left untreated, all patients would develop colorectal cancer) [7]. FAP is genetically characterised by germline mutation in the Adenomatous Polyposis Coli (APC) tumour-suppressor gene and is associated with extracolonic manifestations (GI polyposis, congenital hypertrophy of the retinal pigment epithelium, fibromas, desmoid tumours, dental abnormalities). An attenuated variant (with >10 and <100 colonic polyps) exists. This attenuated variant has a specific hereditary variant called Gardner syndrome (FAP, osteomas, fibromas of the skin and epidermoid cysts). All patients must undergo colectomy at an early age [9].

**MUTYH associated polyposis (MAP)**

MAP is a rare, autosomal recessive hereditary syndrome. It has a similar clinical presentation to attenuated FAP, with polyposis. However, the polyps are not only adenomatous, but also hyperplastic and sessile serrated. MAP is
caused by a biallelic germline mutation in *MUTYH (MYH)*, a DNA base-excision repair gene that acts when DNA damage is caused by oxidative stress [10].

**Hamartomatous polyposis syndromes**

There are many hamartomatous polyposis syndromes, which entail an increased risk of colorectal cancer, but their incidences are very low. The two most common syndromes in this family are:

*Peutz-Jeghers syndrome.* This autosomal dominant syndrome is characterised by the presence of multiple hamartomatous polyps in the small intestine, stomach, and colon. Presenting symptoms are usually obstruction and intestinal bleeding. Common extraintestinal manifestations are pigmentation of the lips and oral and periorbital mucosa. The only known genetic alteration is the *STK11 (LKB1)* mutation, which is involved in the TGFβ pathogenic pathway. These patients have an 81-93% risk of CRC[11].

*Juvenile polyposis syndrome.* This autosomal dominant syndrome presents with juvenile polyps in the GI tract, most often in the colon, with a clinical presentation similar to Peutz-Jeghers syndrome. The associated genetic alterations are mutations in *SMAD4* and *BMPRIA*, which are also involved in the TGFβ pathway. The risk of developing CRC is 39% [12].

**Carcinogenesis in colorectal cancer: From Vogelstein’s tumour model to the present day**

When a neoplasm develops from the epithelium of normal colonic mucosa, with transition into adenomatous epithelium, it presents a well-established adenoma-carcinoma sequence. In 1990, Vogelstein and Fearon proposed a multistep model in tumour carcinogenesis [13]. This model was the starting point for a course of research that has led to today’s knowledge of tumour carcinogenesis triggered by cellular genomic instability.

The loss of genomic stability opens the door to the development of colorectal cancer. Three different types of pathological pathways lead to genomic instability: chromosomal instability (CIN), microsatellite instability (MSI), and the CpG-island methylator phenotype (CIMP). These pathways are not mutually exclusive and therefore more than one may be involved in both sporadic and hereditary development of cancer. The alterations necessary for cancer development are not precisely known, nor are when in
the sequence such alterations must occur, but what is indeed known is the
moment when certain components of these pathways play a major role
[14,15].

Chromosomal instability (CIN)

CIN occurs in 65-70% of sporadic colorectal cancers [16]. CIN is due to
defects in different phases of the cell cycle at a genomic level, which often
lead to the activation of oncogenes and inactivation of tumour-suppressor
genes. The most common mechanisms that cause CIN are: abnormalities in
chromosome segregation at mitosis (with reference to aberrations at
checkpoints as well as centrosomal aberrations and alterations in Aurora and
Polo kinases), telomeric dysfunction (excessive activation of telomerase,
which is very common in CRC), alterations in the machinery responding to
DNA damage (the most characteristic of which are alterations in the p53
gene), and loss of heterozygosity of alleles (through aberrations in
chromosomal recombination, or deletions, for example). Over a hundred
genes are involved in the pathways of chromosomal instability [17]. We will
discuss the most important genes.

\(\text{APC} \rightarrow \text{APC}\) is a tumour-suppressor gene found on chromosome 5q. This
gene is the most frequently mutated (in 80% of adenomas and CRC) and is
considered to be the site of the earliest genetic alterations in the sequence of
carcinogenesis. Its mutation in the germline is responsible for \textit{Familial
Adenomatous Polyposis}. The \text{APC} gene encodes the \text{APC} protein. Among the
latter's many cellular regulatory functions is its responsiblity for the degradation
of the \(\beta\)-catenin protein involved in Wnt stem signaling pathway [18,19].

Degradation of the \(\beta\)-catenin protein is prevented through the aberrant
Wnt pathway, by accumulation in the cytosol and entrance to the nucleus,
where it binds to the lymphoid enhancer factor/T-cell factor (LEF/TCF),
producing an increased transcription of genes that stimulate cell growth and
inhibit apoptosis (such as \text{c-myc, c-jun, Fra-1}). If an \text{APC} mutation prevents
formation of the \text{APC} protein, or causes aberrant protein formation, then
degradation of \(\beta\)-catenin is prevented, thus permitting uncontrolled activation
of the Wnt pathway. Although other alterations occur at other points in this
pathway, the \text{APC} mutation is the most common [20].

\(\text{KRAS} \rightarrow \text{KRAS}\) is an oncogene that mutates in 30-50% of CRCs. These
mutations are thought to be the second alteration in the sequence of
carcinogenesis and occur in precursor lesions of adenomas and carcinomas.
The \text{KRAS} mutations block the action of the enzyme \text{GTPase}, permitting the
uncontrolled activation of the ras cascade, which results in inhibition of apoptosis and in cell proliferation [21].

**TP53** TP53 is a tumour-suppressor gene, known as “the guardian of the genome”. Mutations here are believed to be the third alteration that occurs in the sequence of carcinogenesis, since they appear in the transition from adenoma to carcinoma. The p53 protein is responsible for detecting changes in the DNA (direct damage, aberrant proliferative signals, oxidative stress), which result in putting the cell cycle on hold (cell arrest) in order for these aberrations to be repaired, or to proceed to apoptosis if repair is not achieved. Mutations in *TP53* are universal alterations in human tumours and appear in 50-75% of CRCs [22].

**Others** In addition to mutations in the above genes, many other alterations take place along the sequence. Other oncogenes are activated, such as *BRAF*, *PIK3CA* (related to the Akt/mTOR pathway), and *CTNNB1* (the β-catenin gene); also, inactivation of many tumour-suppressor genes, such as those caused by loss of heterozygosity of 18q, *SMAD2* and *SMAD4* (TGF-β pathway mediators), and *DCC*. Overexpression of COX-2 has also been observed (and the consequent overproduction of PGE2) in the production of proangiogenic factors [22-25].

**Microsatellite instability (MSI)**

MSI occurs in 12-17% of colorectal cancers (3% of which are hereditary) [26]. This pathogenic pathway develops from mutations in the DNA mismatch-repair systems (MMR). Resulting tumours present common clinical features, such as onset in adulthood, occurrence in the proximal colon, and a better prognosis. The presence of this pathway is called the “mutator phenotype” [27].

DNA polymerases sometimes incorporate an incorrect number of bases during replication in long repetitive DNA sequences such as microsatellites. These errors are usually detected by the MMR system, which is responsible for halting and repairing the replication. However, mutation in any component of this system leads to aberrations, resulting in the appearance of abnormal microsatellites. These, in turn, tend to occur in gene-promoter regions that regulate cell growth, in genes that are involved in carcinogenesis (*TGF-β, BAX, Caspase-5, IGF-I, etc*), thus leading to abnormal and uncontrolled cell growth [28].

DNA mismatch-repair systems contain different homologous genes that transcribe the proteins responsible for repairing transcription mismatch
errors. The genes involved are: \textit{MLH1, MLH3, MSH2, MSH3, MSH6, PMS1, and PMS2}. In sporadic forms of MSI, \textit{MLH1} is the most commonly mutated gene, which is silenced by promoter methylation \cite{29}.

Lynch syndrome, or HNPCC (hereditary nonpolyposis colorectal cancer), occurs through the \textit{MSI} pathway in germlines, mainly by mutations in MSH2 and MLH1, which leave a single functioning copy of the protein in each cell. The characteristics of Lynch syndrome will be discussed in more detail later.

\textbf{CPG island methylator phenotype (CIMP)}

DNA methylation is an epigenetic form of regulating gene transcription. In normal physiological function, \textit{CpG islands} (methylation of repetitive sequences between cytosine and guanine) can be found in the promoters of certain genes, leading to their silencing. In the same way, in a pathological process, they provide a pathway for carcinogenesis. Methylation through promoter \textit{CpG islands} leads to the silencing of gene transcription, which happens in tumour-suppressor genes during tumour carcinogenesis, resulting in inactivation (e.g. \textit{p16, MINT1, NEUROG1, MLH1}) \cite{30}.

This pathway has been observed in 24-51\% of colorectal cancers, depending on the detection techniques used \cite{31}. The most significant and frequent methylation occurs on \textit{MLH1} in sporadic colorectal cancers, producing high levels of \textit{MSI}. However, the presence of \textit{CIMP+} together with \textit{MSI-} has been demonstrated to have a worse prognosis. This finding shows that these disease pathways are not mutually exclusive; they coexist within the same tumour, and their different combinations define the individual characteristics of each tumour \cite{32}.

\textbf{Role of the Epidermal Growth-Factor Receptor (EGFR) pathway in colorectal cancer development}

As we have already seen, many molecular pathogenic pathways influence the origin, development, growth, and local and distant invasion of tumours of colorectal origin. This discovery is very important in many practical and clinical aspects and means that we can further our knowledge of the disease and develop specific therapeutic targets. Within this group of pathways, we would like to highlight the \textit{Epidermal Growth Factor (EGF)}, and in particular, the family of receptors to which EGF binds, and the pathways that the receptors activate \cite{33}. 
EGFR

The family of ErbB (HER) receptors - transmembrane receptors associated with tyrosine kinases - is associated with many pathogenic pathways of carcinogenesis in different tumours. These receptors have four subtypes: ErbB1 (HER1/EGFR), ErbB2 (HER2/neu), ErbB3 (HER3), and ErbB4 (HER4) [34].

EGFR activation occurs through ligand-dependent (e.g. EGF, TGFα), ligand-independent (e.g. urokinase, plasminogen), and overexpression mechanisms (in cancer cells, the result is an activation by ligand-independent receptor dimerisation). In all cases, EGFR activation leads to a conformational change in the receptor, with subsequent activation of the associated tyrosine kinase. The activation of EGFR generates the activation of a cascade of multiple associated pathogenic signalling pathways that are involved in cell development, growth, and apoptosis. Alteration of these pathways leads to a state of cell growth and resistance to apoptosis [35].

Uncontrolled activation of EGFR activates the different associated intracellular pathogenic pathways. The RAS-RAF-MAPK pathway is activated, which also occurs through errors in the control of the RAS and RAF oncogenes, leading to cell growth, differentiation, and resistance to apoptosis [36].

Another pathway that is activated is the phosphatidylinositol 3-kinase (PI3K) pathway, which culminates by regulating the activation of AKT/PKB. This pathway is also related to cell growth and survival, by means of proapoptotic protein inactivation. In turn, a suppressor gene, PTEN, negatively regulates this pathway. Mutation of PTEN generates an accumulation of PI3K, making this pathway hyperactive [37].

In addition to these pathways, stress mechanisms activate EGFR and its protein kinase, which in turn activates transcription-factor pathways, such as Protein Kinase C, Jak, and STAT. We know of many different activated pathways, but others are likely yet to be discovered, for the end result is a highly elaborate cascade of activated phenomena, with many intertwined pathways [38,39].

Targeting the EGFR system

Several molecules have been tested against the EGFR pathway, and many more are under development. Some are already available in clinical practice [40].

Cetuximab → This molecule is a monoclonal antibody (IgG1) that binds to the extracellular domain of EGFR causing internalisation and degradation
of the receptor without activating it. The result is the inhibition of EGFR-dependent cell growth [41].

**Panitumumab** This molecule is another monoclonal antibody (IgG2) that also binds to the extracellular domain of EGFR to competitively inhibit other ligands. It prevents EGFR dimerisation, and therefore the EGFR cascade is not activated [42].

**Angiogenesis in colorectal cancer**

New vessel formation is mediated by the balance between pro-angiogenic factors (vascular endothelial growth factor (VEGF), fibroblast growth factors (FGF), platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), and transforming growth factor (TGF)) and antiangiogenic factors (thrombospondin-1, angiostatin, and endostatin) [43]. Neovascularisation that accompanies tumour growth is aberrant and therefore has different characteristics from normal tissue vascularisation. Tumour-induced vessels are fenestrated, i.e., they have pores through which plasma leaks into the extravascular space, with increased interstitial fluid pressure, leading to vessel collapse, greater ischaemia, and acidosis in the tumour tissue. Paradoxically, despite the increased number of vessels, tumour tissues are poorly oxygenated. This tissue hypoxia leads to increased production of pro-angiogenic factors that contribute in turn to generating more vessels, which sets up a cycle favouring further malignant formation. The acidic, hypoxic environment leads to greater genetic and chromosomal instability, therefore enhancing the malignant potential of these tumours. In turn, the fenestration in the newly formed vessels encourages tumour dissemination, because it is easier for the tumour cells to penetrate the new vessels and metastasise at a distance, therefore worsening the prognosis [44,45].

VEGF is the main growth factor involved in angiogenesis in colorectal cancer. It activates different intracellular signalling cascades, leading to endothelial cell growth, migration, differentiation, and enhanced vascular permeability. The VEGF family of growth factors is composed of several proteins encoded by different genes: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGFR-E, and placental growth factor (PIGF). These growth factors are activated when they bind to different tyrosine-kinase-type receptors present in the cell membranes of endothelial cells (VEGFR-1, -2, and -3) [46].

As the tumour grows, the demand for oxygen and nutrients increases, which leads to the intracellular stabilisation of the α subunit of the hypoxia-
inducible factor (HIF-α), thus preventing proteasomal degradation. If HIF-α remains in the cell for a longer time, it binds to the β subunit, forming a HIF-αβ complex that acts as a transcription factor at a nuclear level for many pro-angiogenic factors, including VEGF, FGF, and EGF [47].

We know of three VEGF receptors, which are characterised by having tyrosine-kinase domains in their intracellular portions. Each of these receptors binds with different affinity to the different isoforms of VEGF (-A, -B, -C, -D, -E, and PIGF), playing an important role in tumour angiogenesis, especially in the case of VEGFR-2, which binds to VEGF-A. Also, some coreceptors called neuropilins (-1 and -2) function to increase the affinity of the receptors and their ligands.

Another mechanisms in the regulation of angiogenesis functions independently of VEGF and its receptors. These mechanisms operate through alternative signalling pathways that involve numerous ligands and their receptors, such as angiopoietins and their receptors (tie-2) [48], Ephrin B2 and EphB4 [49], and Delta and Notch [50,51]. Thus, angiopoietin-1 (Ang1) binds to tie-2, activating the PI3K/AKT/mTOR signalling cascade. Ang1 plays a pro-angiogenic role, whereas angiopoietin-2 (Ang2) acts as an antiangiogenic agent. The binding of EphrinB2 and EphB4 stimulates the formation of new capillaries. In the same way, Delta activates the Notch pathway leading to the formation of new vessels. IL-8 is an important factor involved in angiogenesis that appears to regulate angiogenic activation under hypoxic conditions independently of VEGF activation [52]. In fact, patients with stage III CRC who are carriers of a specific IL8 polymorphism (T-251A) overexpress this factor, which implies a worse prognosis [53].

Because angiogenesis is so important in tumour growth and survival, numerous target therapies for inhibiting angiogenesis have been proposed, ranging from anti-VEGF or anti-VEGFR-2 antibodies to receptor tyrosine-kinase inhibitors [54].

Microenvironment in colorectal cancer: Beyond angiogenesis

Precise regulation of intestinal tissue stroma is needed to ensure a perfect balance and permit constant cell renewal. At the base of intestinal crypts there is a specific compartment of stem cells that migrate upwards from the proliferation phase to form enteroendocrine cells in the luminal surface of the digestive tract. The stroma play an important role in establishing a positional gradient to effect signalling and correct binding between the different ligands. Myofibroblasts are the specific stromal cells that perform this process [55].
Stromal regulation is necessary to preserve normal tissue architecture. Myofibroblasts produce a variety of growth factors, prostaglandins, cytokines, and components of the extracellular matrix that facilitate tissue repair and survival [56]. Myofibroblasts are one of the most abundant cell types in the stroma associated with tumour growth. In fact, as the tumour grows, the microenvironment plays an increasingly important part in malignant formation. Cancer-associated or peritumoural myofibroblasts acquire different phenotypic characteristics, increasing production of growth factors and remodelling of proteases in the extracellular matrix, which facilitate tumour migration and invasion [57].

The growth factors in tumour stroma modulates the accumulation of nuclear β-catenin through PDGF. Stromal cells may also provide a suitable microenvironment for the maintenance of tumour stem cells both at the original tumour site and in invasive behaviour and distant migration. The cellular microenvironment therefore acts as a co-effector of a tumour's metastatic ability to increase its malignant potential by providing a suitable substrate for growth. As stromal cells modulate both tumour growth and accumulation of nuclear β-catenin, specific characteristics of these cells can be selected during tumorigenesis so that they continue to provide a suitable microenvironment [58].

In addition to the stromal fibroblasts, other cell types in the peritumoural microenvironment are involved in tumour growth and development. These include T cells. Increased tumour infiltration by these cells should be associated with a better prognosis; however, a subtype of T cell called Tregs that stimulates tumour growth through immunosuppression prevents autoimmunity and permits the growth of commensal bacterial flora. Patients with CRC have an increased number of this cell type in their peripheral blood [59,60].

Other cell types in the microenvironment that may play an important role in tumour growth are cancer-associated macrophages and immature myeloid dendritic cells. All these factors suggest that the inflammatory response is key to tumour survival, extravasation, and metastatic formation. In fact, tumour growth and development depend on the ability of the different stromal factors involved to modulate activity of β-catenin.

Other non-cellular factors in the microenvironment are involved in tumour development and growth, and even in tumour initiation. The hypoxic conditions that characterise tumour progression are associated with the stabilisation of HIF1alpha (hypoxia-inducible factor-1 alpha), which is correlated with a worse prognosis in colon cancer. HIF1alpha binds directly to β-catenin in the nucleus, which is why activation of β-catenin changes under hypoxic conditions [61].
Stem-cell routes involved in colorectal cancer maintenance

The onset of tumorigenesis could occur through the action of a single undifferentiated cell known as a tumour stem cell. These cells are not always destroyed by antitumour treatments and appear to play an important role in resistance to cytotoxic and cytostatic drugs, radiotherapy, and to the new anti-target drugs [62].

Stem cells are undifferentiated cells that undergo asymmetric division to produce two different daughter cells. One daughter cell is identical to the original parent cell, and the other is a more specialised cell. Stem cells maintain their undifferentiated state as a result of this asymmetric division, although they are tied to the different tissues in which they are located and are responsible for maintaining balance and making repairs after any tissue stress [63].

Some studies have shown that errors in DNA replication may occur when stem cells undergo active division, which would mediate in tumour pathogenesis. These tumour stem cells derive from the division of normal stem cells as a result of abnormal differentiation, from stem cells that differentiate directly into tumour cells, or by reprogramming themselves and thus acquiring tumour behaviour.

The colonic crypts of Lieberkühn house the functional unit that produces continuous cell turnover. This complex process is regulated by stem cells located in these crypts. This physical environment is known as the stem-cell niche, where the subepithelial myofibroblasts are located, which are not only responsible for providing the right environment for tumour growth as described in the previous paragraph but are also actively involved in stem-cell division and differentiation through the activation of numerous growth factors. These niches ensure the correct balance between stem-cell division and differentiation [64].

The base of the intestinal crypt is characterised by the activation of the Wnt signalling pathway and is the location of nuclear β-catenin. The majority of sporadic colorectal cancers are caused by the constitutive activation of Wnt due to mutations in the APC suppressor gene or in the β-catenin oncogene, thereby leading to accumulation of nuclear β-catenin. This accumulation leads to the activation of the Wnt pathway and other signalling pathways. We know that nuclear β-catenin binds to different factors, activating several signalling pathways and promoting tumour growth and malignancy [65].

Although the tumour stem-cell theory is based on animal models, which may underestimate the tumourigenic potential of these cells, malignant
transformation is hypothesised to occur in the normal stem cell, resulting in a genetically identical population of tumour cells in which only a few cells retain the characteristics of the original parental cells, thus contributing to tumour progression [66]. On the other hand, the clonal-evolution model hypothesises that any normal cell can be transformed, and that all its offspring can acquire additional mutations, forming a mass of tumour cells with different genetic variations that will promote tumour progression. From a therapeutic point of view, differences depend on the model that is embraced. With the clonal model, the tumour would be heterogeneous, and all its cells would be treated in a targeted manner. However, if the tumour stem-cell theory is embraced, the stem cells would be the therapeutic target since they would be responsible for maintenance and recurrence following the different therapies [67,68].

The main surface markers of intestinal stem cells are Ms1, CD29, Lgr5, and DCAMKL1. Of all the different surface markers expressed on colorectal tumour stem cells, no specific marker has yet been identified, although CD133 may be the most important and most widely expressed in these cells [69]. Data on the expression of this marker, however, are contradictory. ESA, CD44, CD166, Msi1, CD29, CD24, Lgr5, and ALDH1 are other surface markers expressed on colorectal tumour stem cells, and, in fact, during the progression from adenoma to carcinoma, the number of positive cells for CD133, CD44, and ALDH is clearly increased following the gradient in the crypts [70].

**Epigenetics in colorectal cancer: Does it really matter?**

Epigenetic instability appears to play an important role in colorectal carcinogenesis. DNA methylation is an epigenetic mechanism of regulating gene transcription. Aberrant methylation of genes is a mechanism of gene inactivation in patients with CRC[71,72].

DNA methylation is present in much of the human genome in a relatively stable form. A significant number of CpG dinucleotides are carriers of epigenetic modifications, constituting the so-called CpG islands that are normally found in unmethylated form. An aberrant methylation of one of these regions is quite often accompanied by transcriptional silencing. If this aberrant methylation occurs in certain genes, such as MLH1, MGMT, and HIC1, tumour pathogenesis may be affected. In fact, aberrant methylation of MLH1 is found in 80% of sporadic colorectal cancers with microsatellite instability. Aberrant promoter methylation of certain genes (HLTF, SLC5A8, MGMT, MINT1, and MINT31) occurs in the early stages of the adenoma-carcinoma sequence [73,74].
A subset of CRC called CIMP, defined by its high proportion aberrant gene promoter methylation, differs from the CIMP-negative phenotype. However, it is not clear whether these tumours are really a molecular subset of tumours or whether they are a tumour group found in the tail of the normal distribution of all tumours that really present aberrant gene promoter methylation [75].

Most CIMPs present a BRAF mutation, and those that do not have a K-ras mutation [75]. These two mutations therefore appear to be mutually exclusive in these tumours, indicating that this EGFR/BRAF/RAS signalling pathway is critical to the development of these tumours.

DNA methylation can affect transcription. Methylation can affect transcription by directly inhibiting transcription factors and methylation of promoters, but methylation of CpG islands can also activate transcription silencing, by altering binding proteins and histone deacetylases. These altered histones appear to cooperate with DNA methylation in the transcription of tumour-suppressor genes in carcinogenesis [76].

Epigenetic alterations that are commonly observed in CRC are likely to be involved in the development of tumours, activation of oncogenes, and deactivation of suppressor genes. However, the specific mechanisms that contribute to these alterations are currently under investigation, and we do not know the exact role they play in tumour initiation, growth, and maintenance [77].

**Conclusion**

Despite of great efforts performed in last decades, the prognosis of patients with metastatic CRC is poor. Life expectancy is less than 2 years in most of randomized clinical trials. Both the deeper knowledge of the molecular mechanisms involved in pathogenesis of CRC and the improvements of molecular engineering techniques to design new drugs against different targets make this field one of the most attractive in terms of research in solid tumors. Nowadays, there are several new targeted designed compounds under clinical evaluation in colorectal cancer. Systemic treatment of advanced solid tumors in general, and CRC properly, require an adequate individualization. We would need to select the best drug for the right patient according to molecular features that define each tumor.

Therefore, we would not only need more active and safe drugs but also accurate biologic predictors of response to these drugs that will be determined by well established pathology knowledge from the molecular point of view.
References

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