Review

Diet, Microbiota, and Colorectal Cancer

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The intestinal epithelium is a very dynamic tissue under a high regenerative pressure, which makes it susceptible to malignant transformation. Proper integration of various cell signaling pathways and a balanced cross talk between different cell types composing the organ are required to maintain intestinal homeostasis. Dysregulation of this balance can lead to colorectal cancer (CRC). Here, we review important insights into molecular and cellular mechanisms of CRC. We discuss how perturbation in complex regulatory networks, including the Wnt, Notch, BMP, and Hedgehog pathways; and how variations in inflammatory signaling, nutrients, and microbiota can affect intestinal homeostasis contributing to the malignant transformation of intestinal cells.

INTESTINE STRUCTURE AND FUNCTION

The intestine is the last part of the gastrointestinal tract, and is divided into two anatomically and functionally different sections: the small intestine and the large intestine, whose main functions are food digestion, stool compaction, and absorption of water, nutrients, and salts. Both the small and large intestines present an outer layer of smooth muscle with enteric nervous system; a middle layer composed of connective tissue, nerves, and lymphatic vessels; and an inner epithelial layer called the mucosa.

The small intestine presents epithelial folds resembling finger-like protrusions called villi, which face the lumen and aim to maximize the available absorptive area. Villi are surrounded by epithelial invaginations that form the crypts of Lieberkühn (Figure 1). In contrast, the colonic epithelium lacks villi and consists of a plane surface with multiple epithelial invaginations forming the crypts (Clevers, 2013; Lieberkuhn, 1745; Potten, 1995; Simons and Clevers, 2011).

The intestinal epithelium is one of the most rapidly renewing tissues in humans (Potten et al., 1997). Self-renewal is controlled by intestinal stem cells (ISCs) present at the bottom of the crypt. ISCs are +4 leucine-rich repeat-containing G-protein-coupled receptor 5-positive (Lgr5^{+} or Lgr5^{high}). Upon division, ISCs migrate upward to the crypts' upper part and undergo four to five replication cycles to become Lgr5^{-} (Lgr5^{low}) progenitors or transit-amplifying (TA) cells committed to differentiation.

TA cells differentiate and migrate toward the luminal surface until they become terminally differentiated intestinal epithelial cells belonging to two lineages: the absorptive and the secretory. The absorptive lineage includes enterocytes responsible for the absorption of nutrients in the intestinal lumen (Noah et al., 2011). The secretory lineage includes: gobletcells, which secrete protective mucins, and enteroendocrine cells that secrete mucus and hormones such as serotonin and secretin (Barker et al., 2012; Clevers, 2013). Differentiated intestinal epithelial cells continue moving upward to the villus tip, and upon reaching the surface they undergo apoptosis (Simons and Clevers, 2011), allowing the renewal of all the cells in the crypt villus axis (Figure 1).

Importantly, an exception to this upward migration occurs in the Paneth cells, which migrate downward to the bottom of the crypt, where they contribute to the stem cell niche (Simons and Clevers, 2011). Paneth cells are the main source of Wnt3 and EGF signals, necessary for maintaining crypt cells' proliferative capacity, and for the secretion of antimicrobial peptides (i.e., defensins) and hydrolytic enzymes, such as lysozymes, to maintain a sterile environment in the crypts (Figure 1) (Barker et al., 2012; Clevers, 2013; Kim et al., 2005; Sato et al., 2011; Zhao et al., 2007).

Moreover, a population of quiescent cells is present at the +4 position, between crypts' bases and TA cells, known as label-retaining cells, that provide a reserve pool of stem cells if ISCs are depleted or damaged (Barker et al., 2012; Chaves-Pérez et al., 2019; Clevers, 2013).

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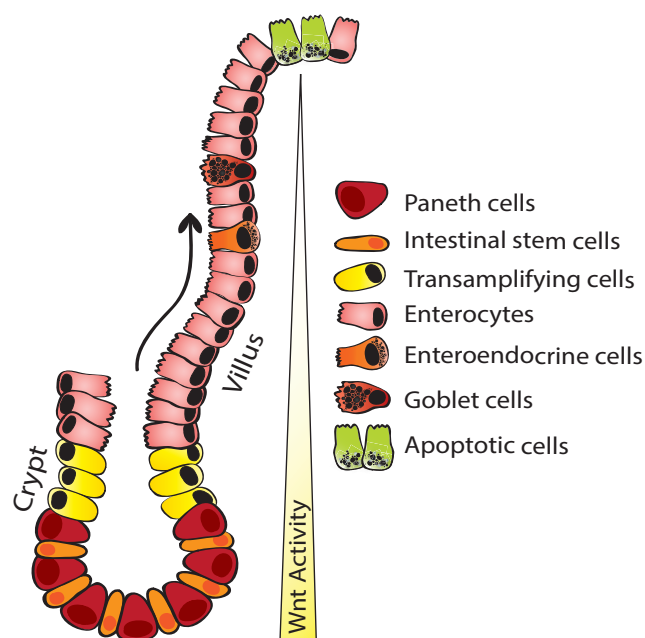
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Figure 1. Intestinal Epithelial Barrier
Schematic representation of the intestinal lining and its cellular components.

REGULATION OF INTESTINAL HOMEOSTASIS

The intestinal epithelium is a very dynamic tissue, where ISCs are under a high regenerative pressure. Thus, a proper homeostatic regulation by a strict balance between cell proliferation, differentiation, and apoptosis is needed (Kitisin and Mishra, 2006). This complex regulatory network involves the Wnt, Notch, BMP, and Hedgehog signaling pathways, which depend on the spatial organization of signals from surrounding mesenchymal cells and the stem cell niche (Medema and Vermeulen, 2011). Deregulation of these main signaling pathways leads to intestinal homeostasis disruption, which contributes to colorectal cancer (CRC) development.

Wnt Signaling Pathway

The Wnt signaling was one of the first pathways described to be implicated in regulating intestinal homeostasis. Current evidence shows that it plays a key role in maintaining crypt stem cell population in proliferating and undifferentiated states (Crosnier et al., 2006). Activation of the Wnt pathway is controlled by the binding of Wnt ligands to LRP5/6 receptors and to receptors from the Fizzled family. The oncoprotein β-catenin is inhibited when bound to the adenomatous polyposis coli (APC). The destruction complex composed by APC, Axin, and two kinases, the casein kinase I (CKI) and the glycogen synthase kinase 3 (GSK3), targets β-catenin for phosphorylation-mediated proteosomal degradation. Wnt binding to LRP5/6 inhibits APC leading to β-catenin stabilization and translocation to the nucleus, where it binds to TCF transcription factors and activates the transcription of Wnt/TCF target genes (Clevers, 2013; Radtke and Clevers, 2005).

The Notch Signaling Pathway

The Notch signaling pathway plays an essential role in controlling intestinal homeostasis. Notch and its ligands are transmembrane protein receptors that mediate cell-to-cell contact. In the intestinal epithelium, Notch signaling plays a crucial role by regulating cell differentiation, determining the lineage fate of TA cells. Activation of the Notch pathway relies on the interaction of five ligands (Jagged1/2 and Delta like-1/3/4) and four receptors (Notch1/2/3/4) (Rallis et al., 2019).

Mechanistically, the interactions of Notch receptors with its cell-bound ligands induce the activation of a proteolytic cascade with three sequential cleavage steps. Upon ligand binding, the S1 site of the Notch receptor is cleaved by a Furin-like convertase forming a Notch extracellular domain, non-covalently bound

to a transmembrane fragment (Logeat et al., 1998). A second cleavage occurs at the S2 site by the action of a metalloprotease from the ADAM family, which gives rise to a transient intermediate peptide that acts as substrate for y-secretase-like protease catalyzing the intramembranous cleavage of the Notch intracellular domain (NICD) at the S3 site (Brou et al., 2000; Mumm et al., 2000; Schroeter et al., 1998; De Strooper et al., 1999). Upon its release, NICD translocates to the nucleus, where it binds to the transcription factor CSL. This complex stimulates the transcription of Notch target genes (Clevers, 2013; Struhl and Adachi, 1998) involved in cell growth, differentiation, angiogenesis, and apoptosis (Al-Hussaini et al., 2011). The role and functions of Notch signaling in CRC has been previously reviewed (Vinson et al., 2016), supporting the role of Notch dysregulation in aberrant cell division, proliferation, and apoptosis resistance, leading to tumorigenesis and metastasis.

BMP Signaling Pathway

The bone morphogenic protein (BMP) belongs to the TGF-β superfamily of ligands that control intracellular signaling through SMAD proteins (Medema and Vermeulen, 2011). In the intestine, BMP2 and BMP4 are expressed in mesenchymal cells, and are responsible for counteracting Wnt signaling and halting proliferation at the crypt-villus border. Importantly, BMPs are active only at the top of the crypt, where they counteract proliferation, and promote cell differentiation.

Mechanistically, BMPs bind to their type II receptor, which leads to the phosphorylation and activation of type I BMP receptor, and subsequently to the activation of SMAD (Gehart and Clevers, 2018). SMAD translocates to the nucleus where it acts either as a co-activator, or as a co-repressor for transcription (Radtke and Clevers, 2005).

The Hedgehog Signaling Pathway

The Hedgehog signaling pathway involves Hedgehog proteins controlling epithelial and mesenchymal cell interaction, as well as BMP production. The constitutive activation of this pathway leads to an increase in the activation of BMP signaling pathway and, therefore, to a reduction in the TA compartment (Van Dop et al., 2009). On the other hand, reduction in the Hedgehog signaling leads to an improper intestinal differentiation and hyperplasia through enhancement of the Wnt signaling pathway (Madison et al., 2005).

COLORECTAL CANCER

Cancer is a multifactorial neoplastic disease, in which both genetic and environmental factors interact affecting proliferation, differentiation, survival, and metabolism of the cells in the organism. The high regenerative pressure, and the constant contact with nutrients and microbiota, makes the intestinal epithelia highly susceptible to malignant transformation.

CRC is one of the most common cancer types. Worldwide, it is currently the third most frequently diagnosed cancer in men and the second in woman. An estimate of 1.7 million cases was reported in 2015, worldwide. In addition, CRC represents the third leading cause of cancer-related deaths, accounting for 9.2% of total cancer deaths worldwide and causing approximately 832,000 deaths in 2015 (Fitzmaurice, 2018).

Risk Factors

Many risk factors have been associated with CRC, and both genetic and environmental factors play a mutual role in tumor development. CRC can have an hereditary or sporadic background, but because CRC has been described as a multi-hit neoplasia, the tumor arises from the accumulation of multiple mutations overtime. For instance, mutations in the tumor suppressor gene *APC* can occur somatically initiating tumor formation, or be a germ line mutation, thus predisposing to tumor development (Vogelstein and Kinzler, 1993).

Hereditary CRC accounts for approximately 5%-10% of all CRC cases and involves inherited mutations in high-risk cancer susceptibility genes (*APC*, DNA MMR genes, p53 mutations, *KRAS*, *BRAF*, among others) (Markowitz and Bertagnolli, 2009). Studies have reported that having one affected first-degree relative increases the risk of developing CRC by 2-fold. This association increases to even 4-fold for individuals with three or more affected first-degree relatives (Kuipers et al., 2015; Taylor et al., 2010). Most common forms of hereditary CRC are linked to familiar CRC syndromes, including hereditary non-polyposis colon cancer

(HNPCC, or Lynch syndrome). These diseases are associated with mutations in the DNA repair pathway, mostly in MLH1 and MSH2, which trigger microsatellite instability (Haggar and Boushey, 2008), and familial adenomatous polyposis (FAP) caused by mutations in *APC* (Nishisho et al., 1991).

The majority of CRC are sporadic and occur due to the accumulation of mutational changes, such as chromosomal and microsatellite instability, that drive the neoplastic process (Kitisin and Mishra, 2006; Vogelstein and Kinzler, 1993). Importantly, many environmental factors have been shown to influence the risk of developing somatic mutations favoring tumor formation (Kuipers et al., 2015). Meta analyses have reported a positive association between CRC and obesity (Renehan et al., 2008), diabetes (Larsson et al., 2005), smoking (Liang et al., 2009), consumption of alcohol and red and processed meat (Martínez, 2005), and dysbiosis (Dahmus et al., 2018).

Preventive factors include physical activity (Samad et al., 2005), aspirin intake (Dube et al., 2007), postmenopausal hormone replacement therapy (Grodstein et al., 1999), calcium (Ca^{2+}) (Cho et al., 2004) and vitamin intake (Song et al., 2015). Moreover, age has been shown to have an influence in CRC incidence as it strongly increases with age, having an estimated median age of diagnosis of 70 years old in developed countries.

Recently, chronic inflammation and inflammatory bowel disease (IBD) have been linked to CRC development. IBD encompasses two inflammation-related conditions of the intestines: ulcerative colitis (UC) and Crohn disease (CD). IBD is characterized by the interaction of different factors such as genetic predisposition, altered microbiota, and environmental factors that trigger an aberrant immune response, leading to impaired intestinal homeostasis. UC is characterized by inflammation of the mucosa of the colon and rectum, whereas CD presents inflammation spread through all the thickness of the bowel wall, affecting all parts of the digestive tract (Haggar and Boushey, 2008).

Mechanisms of CRC

CRC development is characterized by the progressive accumulation of multiple genetic and epigenetic aberrations within cells (Fearon and Vogelstein, 1990; Nguyen and Duong, 2018). In 1990, Fearon and Vogelstein proposed a model for CRC tumorigenesis describing that the total accumulation of genetic and epigenetic mutations was responsible for tumor formation, and its biological properties. In this regard, tumors arise as the result of gradual accumulation of mutations in multiple genes, such as those leading to oncogene activation, or inhibition of tumor suppressor genes (Fearon and Vogelstein, 1990).

However, recent evidences have shown that the progression from polyp to cancer involves not only the accumulation of multiple mutations, but also alteration at different molecular events (Lao and Grady, 2011), and even though the genomic and molecular basis may differ, the conventional pathway for CRC begins as a benign adenomatous polyp that progressively develops into an advanced adenoma with high-grade dysplasia and eventually into an invasive tumor that leads to the loss of the epithelial structure and function. ISCs have been proposed to be at the origin of CRC (Barker et al., 2009; Markowitz and Bertagnolli, 2009) with the significant contribution of micro-environmental factors that support tumor development. Although the sequence of sporadic events that leads to CRC is still poorly understood, it has been well described that the initiating event in CRC is the activation of the Wnt signaling pathway, mainly by mutations in β-catenin, or loss in the *APC* gene, promoting cellular activation and proliferation (Medema and Vermeulen, 2011). Additionally, as further discussed, throughout tumor evolution, adenomas increase microsatellite instability (MSI) and chromosomal instability (CIN), and as adenomas grow, they acquire mutations in the small GTPase KRAS, followed by loss of SMAD4, inactivating mutations in TP53, and loss of PTEN, which together lead to the malignant transformation of the intestinal epithelium (Walther et al., 2009).

Even though generally the malignant transformation occurs from adenoma to CRC, an additional class of premalignant polyps called serrated polyps, with high potential for malignant transformation, is now recognized (Lao and Grady, 2011). In this regard, about 15%–30% of CRCs follow an alternative route of carcinogenesis, called the serrated colorectal carcinogenesis (Yamane et al., 2014). In this model, serrated polyps replace the adenoma as the precursor lesion progressing to CRC. Serrated polyps originate upon BRAF mutations, and hypermethylations in the promoter area of the CpG islands of tumor suppressor

genes (Villanacci et al., 2019). Importantly, in the serrated pathway the methylation and inactivation of DNA repair genes (such as MLH1 and MGMT), leading to DNA damage, has been described as an important step leading to genetic instability (Jass, 2005). Low levels of CIN are enough to lead to genetic variations and, together with interleukin (IL) 6 infiltration can promote CRC in a Wnt-independent matter (Brandt et al., 2018; Jass, 2005).

Genomic Instability

Chromosomal Instability

CRC is a very heterogeneous disease, and its development involves multiple molecular pathways where genomic instability, mutational inactivation of tumor suppressor genes, and activation of oncogenes play the most important role (Markowitz and Bertagnolli, 2009). Genomic instability can promote the development of CRC by facilitating the acquisition of multiple tumor-associated mutations (Brandt et al., 2018; Markowitz and Bertagnolli, 2009). Among them, CIN is present in about 80% of all CRC and leads to multiple changes in chromosomal copy number and structure, resulting in karyotype variability from cell to cell (Lengauer et al., 1997). As a consequence, CIN leads to imbalanced chromosome number (aneuploidy or polyploidy), chromosomal rearrangements, and loss of heterozygosity (LOH) (Pino and Chung, 2010). Most importantly, these alterations may affect the expression of important tumor-suppressor genes, such as *APC*, *TP53*, and *SMAD4* (Markowitz and Bertagnolli, 2009).

Microsatellite Instability

Microsatellites are short DNA motifs (of 1–6 bases) of tandem repeats, distributed through the genome in both coding and non-coding regions (De'Angelis et al., 2018). Data derived from the observation of mutations at simple repeated sequences, in sporadic colorectal tumors (Ionov et al., 1993; Thibodeau et al., 1993) and HNPCC tumors, lead to the study of MSI relationship with tumorigenesis (Aaltonen et al., 1993). Further studies described that the intrinsic somatic instability derived from these mutations at microsatellite-associated genes contributed to the acquisition of tumor-like phenotype and tumor malignancy; and that these alterations are mainly due to defects at early steps of the strand-specific mismatch repair (Ionov et al., 1993; Parsons et al., 1993; Thibodeau et al., 1993).

Indeed, MSI involves the inactivation of mismatch repair (MMR) genes required for DNA repair (Markowitz and Bertagnolli, 2009). Owing to the repetitive structure of microsatellites, replication errors are common and normally repaired by MMR proteins (De'Angelis et al., 2018; Vilar and Gruber, 2010). Loss of function of MMR genes leads to impaired repair machinery and therefore, to the accumulation of mistakes in the microsatellites (Gelsomino et al., 2016). Importantly, tumor suppressor genes that have functional regions containing mononucleotide or dinucleotide repeated sequences can also be inactivated (Markowitz and Bertagnolli, 2009).

This inactivation has been widely described to occur in cancerous cells; in fact, MSI occurs in >95% of HNPCC, and involves germ-line mutations in one or more of the MMR genes, such as MLH1, MSH2, MSH6, and PMS2; and in 15%–20% of sporadic CRC, caused mainly by epigenetic inactivation of the MLH1 gene (Gelsomino et al., 2016; Nguyen and Duong, 2018).

Aberrant Methylation

Aberrant methylation of DNA is a third mechanism triggering genomic instability, and it is present in about 15% of CRC cases. DNA methylation is the process in which a methyl group is attached to the 5′-position of cytosine by DNA methyltransferases to produce 5-methylcytosine (Lao and Grady, 2011). In normal conditions, cytosine methylation occurs in repetitive DNA sequences called CpGs, and only CpG islands in promoter regions are unmethylated (Markowitz and Bertagnolli, 2009). Aberrant methylation is common among the CRC genome, leading to the hypomethylation or hypermethylation of CG dinucleotide sequences (GpGs). Aberrant hypermethylation of CpG islands within the promoter region leads to silencing of many tumor suppressor genes (Toyota et al., 1999). In fact, genomic studies have shown that 1%–10% of CpGs islands are hypermethylated, and tumor suppressor genes such as CDKN2A, MLH1, CDH1, and VHL are affected, contributing in the tumorigenic process (Lao and Grady, 2011). On the other hand, the inverse effect is observed in the loss of DNA methylation, leading to the overactivation of oncogenic genes, and genomic instability, favoring tumorigenesis (Lao and Grady, 2011).
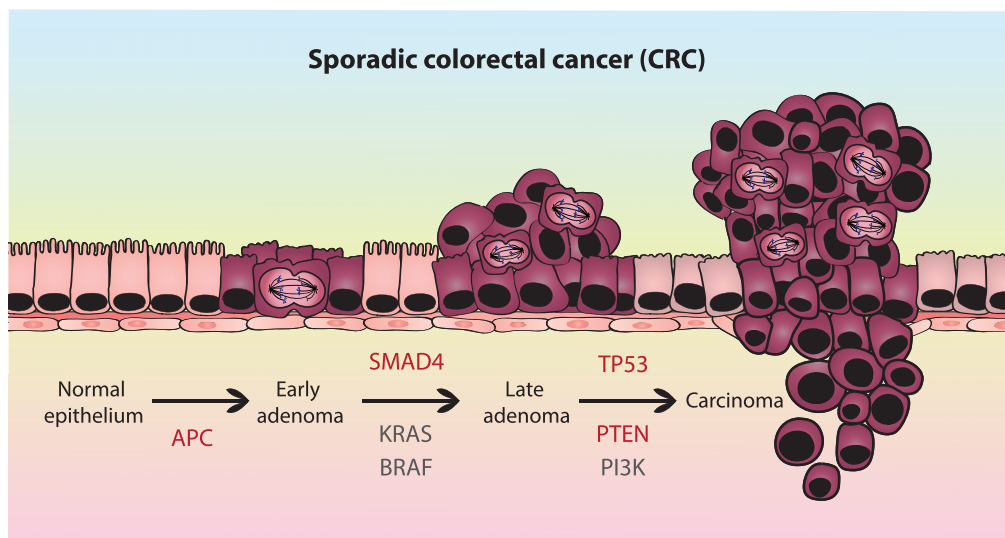
Figure 2. Stepwise Events in Sporadic Colorectal Cancer Development
Schematic representation of events leading to carcinogenesis of sporadic CRC. Red represents gene loss, whereas gray represents genes and cytokines activated during cancer progression.

Inactivation of Tumor Suppressor Genes

Mutations in the APC Complex. The sequence of sporadic events that lead to CRC is still poorly understood. A wide range of evidences suggests that the initiating event in CRC is the activation of the Wnt signaling pathway, mainly by mutations in β-catenin, or loss of *APC* (Figure 2). As previously described, the APC complex acts as a negative regulator of the Wnt signaling pathway. Mutations in *APC* disrupts the formation of the APC complex, leading to the translocation of β-catenin to the nucleus and, therefore, to the over activation of the Wnt signaling, promoting cellular activation and proliferation (Clevers, 2013; Markowitz and Bertagnolli, 2009; Nguyen and Duong, 2018; Walther et al., 2009).

APC is mutated in 80%–90% of hereditary and spontaneous cases of CRC (Perochon et al., 2018). Clearly, germline mutations in *APC* are responsible for FAP (Bodmer et al., 1987), whereas 80% of all spontaneous forms of CRC present somatic mutations in APC (Fearnhead et al., 2001) Around 60% of these somatic mutations occur within the mutation cluster region (MCR) of the APC gene, affecting its binding to Axin or β-catenin (Miyoshi et al., 1992; Perochon et al., 2018). Furthermore, and less frequently, activating and stabilizing mutations in the β-catenin (*CTNNB1*) gene (Samowitz et al., 1999) have been implied in CRC. Mouse research has shown that *Apc* loss can lead to adenoma formation, and together with KRAS and p53 mutations, adenomas progress into aggressive carcinomas (Dow et al., 2015). Importantly, restoration of APC leads to tumor suppression via Wnt suppression, pointing out the importance of APC restoration in CRC treatment (Figure 2) (Dow et al., 2015).

Mutations in TP53. *TP53* regulates the expression of genes involved in cell cycle progression, cell-cycle arrest, DNA repair, and apoptosis (Kuipers et al., 2015). *TP53*, located in the chromosome 17p, is the second most frequently inactivated tumor suppressor gene, and *TP53* mutations are present in about 50% of all CRC cases (Baker et al., 1989; Pritchard and Grady, 2011). Upon 74% of *TP53* alterations are due to missense mutations in the "hot spot" residues of the DNA-binding domain, leading to the loss of its transcriptional activity, or to the gain of p53 oncogenic functions (Nakayama and Oshima, 2018), promoting, therefore, the malignant transformation of adenomas into invasive carcinomas.

Interestingly, recent evidences have shown that mutations in *TP53,* leading to its dysfunction, can modulate the gut microenvironment and promote inflammation and immune evasion, favoring tumor progression (Guo et al., 2017). Moreover, cells harboring p53 mutations, leading to the gain of oncogenic functions, have shown to modulate macrophage function increasing pro-tumorigenic factors secretion and promoting tumor progression (Cooks et al., 2018). Importantly, recent studies have shown that targeting and

activating pharmacologically p53 stimulates the activity of the systemic adaptive immune system favoring tumor regression and suppression (Guo et al., 2017).

Mutations in the TGF-β Pathway. Finally, deregulation of the transforming growth factor β (TGF-β) pathway, responsible for mediating growth arrest and apoptosis, is affected in about one-third of CRC cases (Markowitz and Bertagnolli, 2009). Inactivating mutations in receptor genes, such as *TGFBR2* and *TGFBR1*, and in genes involved in the downstream signaling (such as SMAD2 and SMAD4) have been observed. In addition, the depletion of the long arm of chromosome 18 (18q) containing TGF-β pathway mediators, such as SMAD2 and SMAD7, lead to the most common cytogenetic abnormalitys in CRC (Pritchard and Grady, 2011).

Activation of Oncogenes

Mutations in Kras Signaling Pathway. Several oncogenes play an important role in promoting CRC. *KRAS*, a member of the RAS family of proto-oncogenes, occurring in approximately 40% of CRC cases (Pritchard and Grady, 2011). Usually, mutations occur in the codons 12 and 13 of the *KRAS* gene (Vogelstein et al., 1988), resulting in the constitutive activation of KRAS. Upon its activation, KRAS signals downstream of the epidermal growth factor receptor (EGFR) to activate the mitogen-activated protein kinase (MAPK) pathway, promoting cellular growth and survival (Pritchard and Grady, 2011). Upon KRAS mutations, the EGFR pathway is over-activated, leading to an increase in stem cell proliferation (Gehart and Clevers, 2018; Snippert et al., 2014), favoring tumor formation and progression.

Furthermore, mutations in *KRAS* lead to immune suppression through IRF2, favoring tumor progression (Liao et al., 2019), and promote metastasis together with Tp53 mutations (Nakayama and Oshima, 2018) . Importantly, KRAS has become an important target for tumor treating, and several studies aim to identify molecules capable of binding and inhibiting KRAS biological activity (Porru et al., 2018). Among them, the small non-coding RNA miR-16 has been identified to directly regulate and silence KRAS, leading to the in-hibition of proliferation and invasion of cancer cells, and favoring apoptosis. Xenograft mouse model studies validated the tumor-suppressive role of miR-16 (You et al., 2016). AZD4785 is another promising high-affinity therapeutic antisense oligonucleotide inhibitor that binds to, and inhibits KRAS and its down-stream effectors, reducing, therefore, cellular proliferation and showing antiproliferative and antitumoral effects (Porru et al., 2018).

Another oncogene frequently mutated in CRC is *BRAF*. *BRAF* encodes for a protein kinase, which is a direct downstream effector of KRAS in the Ras/Raf/MAPK signaling pathway (Pritchard and Grady, 2011). The constitutive activation of BRAF due to point mutations leads to the activation of the MAPK signaling cascade leading to the activation of several transcription factors that promote cell survival, proliferation, and metastasis (Rajagopalan et al., 2002). Point mutations in the *BRAF* gene, located in chromosome 7, are present in about 10%–15% of CRC cases (Pritchard and Grady, 2011). BRAF V600E mutation accounts for 90% of BRAF-positive cases, and its presence correlates with poor prognosis and median survival, when compared with BRAF wild-type CRC cases (Luu and Price, 2019). Importantly, KRAS and BRAF mutations are mutually exclusive, and it is unlikely that both mutations occur in the same tumor, although mutations in any of these genes have an equivalent effect in tumor formation by promoting tumorigenesis through increased MAPK activity (Rajagopalan et al., 2002).

Mutations in the PI3K Pathway. Phosphatidylinositol 3-kinase (PI3K) pathway plays a fundamental role in the regulation of cell growth, metabolism, and survival (Engelman et al., 2006). Importantly, the PI3K pathway has been found to be dysregulated in almost all human cancers, and approximately 40% of CRC have impairments in this circuit (Samuels et al., 2004; Yang et al., 2019). Most commonly, mutations affecting the PI3K pathway occur in the p110α catalytic subunit of *PIK3CA*, promoting the transition from adenoma to carcinoma (Samuels et al., 2004). Inactivating mutations in tumor suppressor genes such as the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) are also observed. PTEN acts as a negative regulator of the PI3K pathway, and its loss or downregulation results in the hyperactivation of the PI3K signaling pathway (Waniczek et al., 2018).

Regulation of PI3K/PKB/AKT activity by PTEN is also critical for the activity of the nutrient sensing pathway mammalian/mechanistic target of rapamycin complex 1 (mTORC1), a 290-kDA-serine/threonine protein ki-nase belonging to the phosphoinositide 3-kinase (PI3K)-related kinase family (Zhang et al., 2009).

Importantly, the PI3K pathway has been shown to be dysregulated in CRC patient samples, presenting elevated protein levels, point mutations of PI3K and AKT, and inactivating mutations in PTEN (Johnson et al., 2010; Wang and Zhang, 2014).

NUTRIENTS AND CRC

Activation of Nutrient Sensing mTORC1 Pathway

mTORC1 Pathway

Dietary habits, such as malnutrition, obesity, and diabetes are among the top risk factors that predispose to IBD and CRC. Growth Factors (GFs), nutrients, cellular energy status, and stress signals such as hypoxia and glucose deprivation are upstream signals that regulate mTORC1 activity (Fawal et al., 2015). mTORC1 deregulation leads to an impairment of cellular homeostasis prone to enhance tumorigenesis.

The integration of GF and nutrient signals is essential for mTORC1 activity. In response to GF, such as insulin and IGF-1, mTORC1 is activated through the PI3K signaling pathway leading to the activation of AKT (Mossmann et al., 2018). Once active, AKT regulates mTORC1 activity through the inhibition of two independent substrates, PRAS40 and TSC2. PRAS40 acts as a negative regulator of mTORC1 when associated with RAPTOR, a regulatory-associated protein (Liko and Hall, 2015). PRAS40 phosphorylation by AKT results in its dissociation from mTORC1 (Hay and Sonenberg, 2004), activating mTORC1. Moreover, AKT signals through the tuberous sclerosis complex TSC1/2, which acts as a GTPase-activating protein (GAP) for the Ras homologue enriched in brain (Rheb) GTPase (Fawal et al., 2015). mTORC1 is regulated and activated by Rheb in its GTP form; in the absence of GF, TSC2 inactivates Rheb by hydrolyzing GTP and keeping it in its inactive GDP-bound state (Garrido et al., 2016). Upon TSC1/2 inhibition, GTP-bound Rheb binds to and activates mTORC1 at the lysosomal surface. Importantly, amino acids (AA), mostly leucine and arginine, are essential for fully activating mTORC1 (Fawal et al., 2015). In an AA-dependent manner, inactive mTORC1 (present in the cytoplasm) is recruited to the lysosomal surface, where together with the microsphere protein 1 (MCRS1) it binds to Rheb and activates mTORC1 (Fawal et al., 2015). Upon AA depletion, MCRS1 and Rheb interaction is reduced, and Rheb becomes exposed to the inhibitory activity of TSC1/2, resulting in its delocalization from the lysosome and subsequent deactivation of mTORC1 activity (Fawal et al., 2015). In addition, other stimuli such as inflammation, Wnt signaling, low energy status, hypoxia, and DNA damage can influence the TSC1/2 activity affecting mTORC1 signaling (Jewell and Guan, 2013).

Once active, mTORC1 regulates important cellular processes, such as protein, nucleotide, and lipid synthesis; cellular metabolism; ATP production; and autophagy, aiming to maintain an appropriate balance between anabolic and catabolic processes (Figure 3) (Laplante and Sabatini, 2012). Importantly, mTORC1 regulates the activity of the translational machinery that promotes cell growth and proliferation through phosphorylation of the ribosomal protein S6 kinase (S6K) and the eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4EBP) (Sengupta et al., 2010). Additionally, mTORC1 plays an important role in lipid biosynthesis through SREBP 1/2, increases the glycolytic flux by activating the transcription and translation of the hypoxia inducible factor 1α (HIF1α), regulates the expression of genes involved in oxidative metabolism, and promotes growth by inhibiting autophagy and lysosome biogenesis (Laplante and Sabatini, 2012). mTORC1 is thus a key sensor of nutritional cues and plays critical functions in maintaining intestinal epithelial homeostasis. Moreover, mTORC1 activity in Paneth cells can regulate ISCs self-renewal and differentiation in response to nutrient availability (Yilmaz et al., 2012).

Oncogenic Role of mTORC1 in CRC

Many oncogenes involved in CRC elicit part of their oncogenic function through the mTOR-signaling pathway. Several upstream oncogenic pathways activate mTORC1 (Wang and Zhang, 2014). Rab1A overexpression promotes mTORC1 activity, correlating with poor prognosis (Thomas et al., 2014). Moreover, activation of mTORC1 downstream effectors is increased in CRC and correlates with a higher malignancy (Zhang et al., 2009). Because mTORC1 plays an important role in CRC development, it has emerged as a potential target for drug development. Animal studies performed in APC mutant mice have shown that mTORC1 inhibition, using RAD001, inhibits intestinal polyp formation (Fujishita et al., 2008) and its genetic inhibition reduces tumor incidence (Brandt et al., 2018; Faller et al., 2015). Rab1A, another mTORC1 activator, has also been shown to be upregulated in CRC and correlated with a poor prognosis (Thomas et al., 2014).
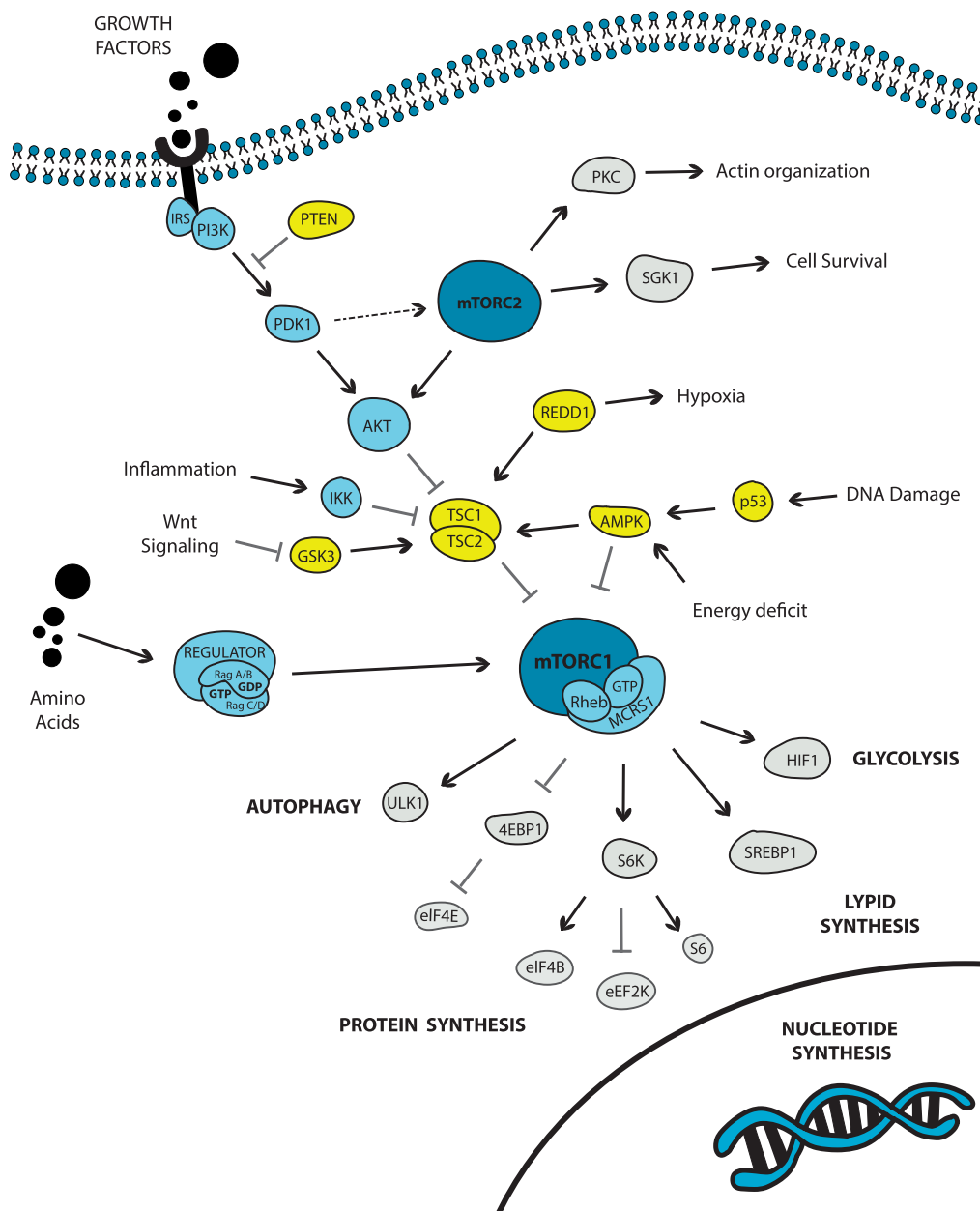
Figure 3. mTOR Signaling Pathway

Tumor Suppressive Role of mTORC1 in CRC

On the other hand, genetic ablation of mTOR in the intestinal epithelium leads to an altered epithelial morphology, impaired differentiation, and aberrant regeneration (Sampson et al., 2016). Additionally, genetic ablation of Rheb or rapamycin treatment impairs intestinal regeneration after acute DSS treatment (Guan et al., 2015). In line with these findings, mTORC1 inactivation induces DNA damage and triggers CIN in IBD models leading to CRC development (Brandt et al., 2018), and its inhibition increases mucosa damage, enhances ulceration, increases immune cell infiltration, and stimulates tumor formation in colitis-associated cancer (CAC) (Gutiérrez-Martínez et al., 2019). In mouse models of colitis-induced CRC, ablation of Regnase-1, involved in immune response regulations, potentiates mTORC1 signaling and reduces colitis

and tumor progression (Nagahama et al., 2018). Furthermore, activating mTORC1 by overexpression of MCRS1 or a high-protein diet shows a protective role in colitis-induced CRC mouse models (Brandt et al., 2018). Recent human data analysis by tissue microarrays showed a negative correlation between MCRS1 expression and inflammation markers (CD68), suggesting that mTORC1 inhibition may be associated with an increased risk of inflammation in a CAC model. Increased MCRS1 and mTORC1 activation correlates with low inflammation but positively with β-catenin (Brandt et al., 2018). Given the fact that most human CRCs are due to environmental factors most likely progressing to IBD, these findings may explain why the overall responses observed in clinical trials using mTORC1/2 inhibitors are very modest. Further studies are necessary to better understand mTORC1 dual role in CRC development and progression.

Diet, Nutrition, and CRC

CRC incidence is increasing among individuals younger than 50 years and seems to be associated with specific dietary factors that affect the gut microbiota, leading to dysbiosis. Preclinical and clinical studies have linked the consumption of some foods and nutrients to an increased or decreased risk of CRC development. Among them multiple nutrients, whole grains, fibers, vitamins, dairy products, and calcium have been linked to a lower risk of CRC (Song et al., 2015), whereas red and processed meat, alcohol, and low levels of micronutrients may lead to an increase in the susceptibility to develop CRC (Martínez, 2005). However, the potential dietary contribution in CRC still remains controversial, and more studies are required to fully elucidate its role.

Calcium (Ca^{+2}) supplementation reduces CRC risk in mouse models (Cho et al., 2004) by inhibiting the proliferation, differentiation, and apoptosis of cancer cells, and inhibits oxidative DNA damage (Fedirko et al., 2010). Additionally, vitamin D has also shown anticancer activities (Song et al., 2015) since its active form regulates multiple signaling pathways involved in proliferation, apoptosis, differentiation, inflammation, invasion, angiogenesis, and metastasis (Feldman et al., 2014). Though, studies performed in animal models support the role of Ca^{+2} and vitamin D in tumor suppression, clinical trials do not fully support its beneficial role. In this regard, Bolland et al. described a non significant reduction of 17% in CRC risk, in woman treated for 7 years with 1 g of Ca^{+2} (Bolland et al., 2011). Moreover, meta-analytic studies of CRC incidence and mortality showed that vitamin D significantly reduces cancer mortality, but not its incidence (Keum and Giovannucci, 2014). In line with these findings, a randomized, double-blind, placebo-controlled clinical trial showed that daily supplementation with vitamin D (2,000 IU) together with Omega3 (1 g) did not lower the incidence of tumor development (Manson et al., 2019). Similar studies involving postmenopausal woman showed that daily Ca^{+2} (1 g) and vitamin D (400 IU) supplementation for 7 years did not affect CRC incidence, and tumors did not differ in their histological characteristics (Wactawski-Wende et al., 2006). On the other hand, the use of higher doses of Ca^{+2} (1.4–1.5 g) and vitamin D (1,100 IU) did reduced the incidence of all cancer types, including CRC (Lappe et al., 2007). Although the therapeutic effects of vitamin D in CRC still needs to be demonstrated, most of the clinical trials performed in healthy patients were aimed to be preventive. Moreover, the importance of personalized therapy relies in the fact that tumor behavior is completely heterogeneous, and resistance or sensitivity to therapies may vary among patients. Having this in consideration, a better approach to clinical studies may be studying the role on tumor progression, stratifying patients, and taking into account the genetic background and lifestyle of patients to subdivide the population that may benefit by each individual treatment. Further studies are thus still needed to fully elucidate the role of Ca^{+2} and vitamin D suplementation in human CRC.

Multiple vitamin B forms, such as folate (B9), riboflavin (B2), pyridoxine (B6), cobalamin (B12), and AA, such as methionine, are essential for DNA methylation, synthesis, stability, and repair (Song et al., 2015), and are therefore believed to play a beneficial role in maintaining intestinal homeostasis. Among them, folate has shown to have a protective role against the development of CRC (Martínez, 2005), and both randomized clinical trials and cohort studies have shown the beneficial effects of folate in the primary prevention of CRC (Moazzen et al., 2018). On the other hand, folate has also been implied as a promoting factor by inducing the growth and progression of preexisting neoplasms (Choi and Mason, 2002), proving again the importance of differential treatments during different stages of tumor initiation and progression.

Fruits and vegetables are known for their antioxidant properties. Vitamin A, C, and E have been shown to have potent anti-oxidative and anti-inflammatory effects (Song et al., 2015). Importantly, clinical trials, including histologically confirmed patients with CRC and controls with acute non-neoplastic conditions, showed that the total antioxidant capacity is inversely corelated with CRC risk, proving the reduced risk

of tumor development upon vitamin supplementation (La Vecchia et al., 2013). In the same way, *in vitro* studies showed that human CRC cells with mutations in *Kras* or *Braf* are selectively killed when exposed to high levels of vitamin C, and *in vivo* studies using *Apc/Kras* mutant mice show tumor growth impairment upon vitamin C supplementation (Yun et al., 2015).

Previous studies have focus on the role of single nutrients in tumor development. Although these studies show an important relationship of individual nutrients, they do not take into account nutrient interaction, and the complexity of the diet itself (Hu, 2002). Therefore, dietary pattern analysis defining the micro- and macronutrients beneficial or harmful for the intestine may be a better approach in nutrient biology (Bravi et al., 2010). Moskal et al. identified four nutrient patterns including PC1, which presents a high nutrients content derived from plant sources and a low content from animal sources; PC2, which includes a high content of micro-nutrients and proteins; PC3, which includes polyunsaturated fatty acids and vitamins; and PC4, which is characterized by a high content of calcium, proteins, riboflavin, and phosphorus in the diet (Moskal et al., 2014). The cohort study implied 477,312 patients, and aimed to understand the potential contribution of dietary patterns in CRC predisposition and ocurrence. Moskal et al. identify that both PC2 and PC4 were inversely associated with CRC risk; and observed a 10% reduction in the overall risk upon a PC4 diet, suggesting the possible role of folate and vitamin B12 in a lower CRC risk, and the presence of calcium with antiproliferative and antitumorigenic effects (Moskal et al., 2016). Consistently, Bravi et al. described three dietary patterns, including diets rich in starch, diets rich in vitamins and fiber, and diets rich in unsaturated fats (from both animal and vegetable sources), and identified that starch-rich diets act as an unfavorable risk predisposing to colon and rectal cancer, whereas vitamins and unsaturated fats were associated with a reduced risk of CRC (Bravi et al., 2010). These studies may represent an important view of the effects of overall diet, therefore representing a broader picture of food and nutrient interactions.

MICROBIOTA AND CRC

The gastrointestinal tract is densely populated by various microorganisms, and bacteria is the most common microbe present in the gut mucosa. Metagenomic analysis have shown that human microbiota contains close to 1,000 to 1,150 different bacterial species, and that each individual may contain approximately 160 different species (Qin et al., 2010). Commensal microbiota play an important role in maintaining intestinal homeostasis, and have been implicated in the regulation of tissue development and maintenance of the mucosal barrier integrity (Natividad and Verdu, 2013). Furthermore, they are involved in several metabolic processes, providing nutrients and vitamins; and generating short-chain fatty acids critical in energy balance of the host cells (Thursby and Juge, 2017). In addition, they have been involved in the modulation of the hosts' immune response, inflammatory cascade, and protection against pathogens (Lee and Mazmanian, 2010).

Although the presence of bacteria usually occurs in a symbiotic background, accumulated evidence has linked bacterial contribution in carcinogenesis (Garrett, 2015), and therefore, dysbiosis due to microbiota dysregulation might trigger the malignant transformations of colon cells. Moreover, the colon contains approximately 1 million-fold higher levels of microbiota when compared with the small intestine, and CAC development is almost 12-fold higher in this organ, pointing out the possible role of bacteria and fungi in tumor development (Proctor, 2011). Metagenomic analysis have shown that species such as *Fusobacterium nucleatum, Bacteroides fragilis, Escherichia coli,* and *Clostridium* are transcendentally abundant in the CRC microbiome and present virulent and toxic mechanisms that might play a critical role in CRC (Wirbel et al., 2019).

For instance, *F. nucleatum*, a rare microbiome in the gut of healthy people, is significantly increased in patients with adenomas and adenocarcinomas, and its presence could potentiate tumorigenesis and modulate the tumor-immune microenvironment (Kostic et al., 2013). *F. nucleatum* is capable of attaching and invading the colonic epithelium through the FadA adhesion protein (Rubinstein et al., 2013). FadA binds and forms a complex together with E-cadherin and Annexin A1, which enhance the activation of the β-catenin signaling and, therefore, the overexpression of its transcription factors, Wnt genes, oncogenes, and inflammatory genes (Rubinstein et al., 2013). In addition, *F. nucleatum* can bind to inhibitory receptors such as TIGIF and CEACAM1, suppressing immune cell activity (Gur et al., 2019). Importantly, treating Fusobacterium-positive colon-cancer xenografts with metronidazole, an antibiotic to which Fusobacterium is sensitive, not only decreases the Fusobacterium load, but also significantly reduces tumor cell proliferation and tumor growth (Bullman et al., 2017).

Interestingly, some commensal bacteria may play a dual role in tumor formation. The colonic bacterium *B. fragilis* has two molecular subtypes: the enterotoxigenic strain (ETBF), and the nontoxigenic strain (NTBF). ETBF produces the *B. fragilis* toxin (BFT), a metalloprotease that binds and cleaves E-catenin, disturbing epithelial cell permeability and barrier function (Wu et al., 1998), and triggers β-catenin nuclear localization, increasing c-Myc expression and cellular proliferation (Wu et al., 2003). Importantly, *B. fragilis* has also been identified as a trigger for colitis and tumorigenesis by promoting an aberrant immune response through IL-8 and IL-17 production (Chung et al., 2018; Sanfilippo et al., 2000; Wu et al., 2009). On the other hand, the NTBF strain has been shown to have a protective role against CRC, acting as an anti-inflammatory probiotic, protecting against *H. hepaticus*-induced colitis (Mazmanian et al., 2008), and inhibiting inflammation in the DSS model (Lee et al., 2018). Additionally, NTBF has a protective role against colon tumorigenesis induced by ETBF (Chung et al., 2018) and AOM/DSS (Lee et al., 2018).

Likewise, bacterial metabolites and their by-products may impact positively or negatively in tumorigenesis. Fermentation products such as butyrate, acetate, and propionate have a positive correlation with the suppression of inflammation and cancer (Louis et al., 2014). However, other bacterial by-products such as colibactin from *E. coli*, cytolethal distending toxin, produced by several Gram-negative bacteria, and hydrogen sulfide, produced by sulfidogenic bacteria, have genotoxic properties that lead to DNA damage and CIN (Dahmus et al., 2018; Guerra et al., 2011; Helmink et al., 2019), which are key factors for tumor initiation and progression.

Importantly, the gut mucosa spatially segregates the gut microbiota and the host's immunity to avoid unwanted immune responses to commensal microbes. Impairment of the mucosal barrier allows the gut bacteria to invade the mucosa inducing an excessive immune response that leads to nuclear factor (NF)-κB activation and intestinal inflammation (Garrett, 2015; Okumura and Takeda, 2018). Excessive and chronic inflammation predispose to CAC, one of IBD characteristics.

Further studies have shown that microbiota plays an important role in cancer therapy outcome. Recent data using genetic and chemical CRC mouse models suggest that CRC development can be modulated by housing mice in germ-free environments, co-housing them with wild-type counterparts or by antibiotic treatment (Fichtner-Feigl et al., 2015; Lasry et al., 2016). Additionally, cancer treatment can promote two dysbiosis states: a detrimental dysbiosis, which limits drug therapeutic efficacy, or increases its toxicity; or a beneficial dysbiosis state, which improves the drug clinical activity (Zitvogel et al., 2015). Therefore, microbiota modulation may prevent and ameliorate CAC and CRC progression or improve its response to cancer treatments.

ROLE OF MICROENVIRONMENT

Many molecular pathways are involved in CRC development, and different levels of evidence support the role of microenvironment in tumor formation. It has been shown that chronic inflammation in patients with IBD predisposes to tumor formation. CAC, linked with IBD, is therefore being recognized as a subtype of CRC (Brandt et al., 2018; Haggar and Boushey, 2008; Terzić et al., 2010). Interestingly, most of the key developmental stages of CAC are shared with the conventional pathway of CRC tumorigenesis including: aberrant crypt foci and polyp formation, and transition from adenoma to carcinoma (Figure 4). Alternatively, CAC can develop from the progression of dysplasia into carcinoma (Lasry et al., 2016).

In addition, alterations in genetic and molecular signaling pathways are common for both CRC and CAC, although the timing may differ. Although genetic risk factors are not present, mutations in *APC*, p53, and KRAS usually occur at later time points of CAC development, when compared with sporadic CRC (Brandt et al., 2018; Terzić et al., 2010). Interestingly, constitutive activation of transcription factors involved in the inflammatory response pathway, such as NF-κB or STAT3 is observed, pointing to the importance of inflammation in CRC development.

CRC can be infiltrated with various types of immune cells that contribute to either tumor immunosurveillance or tumor promotion. Although it is unlikely that inflammation initiates sporadic CRC, it has been shown that pro-tumorigenic immune cells are recruited during tumor formation favoring CAC initiation (Terzić et al., 2010). In CAC, chronic inflammation favors oxidative damage and increases reactive oxygen species, inducing mutations in p53 and MMR genes. Additionally, chronic inflammation
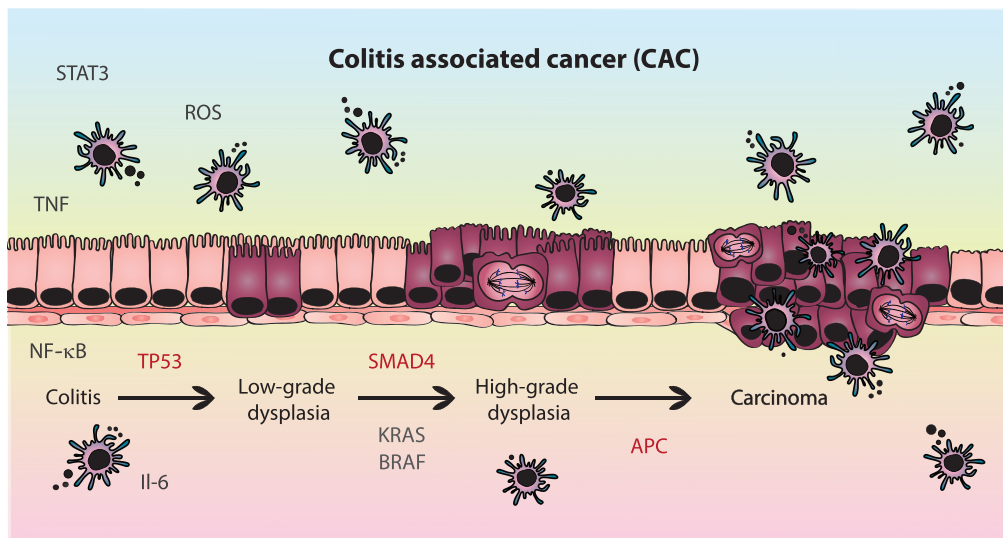
Figure 4. Sequence of Genetic and Inflammatory Events that Occur during Colitis Associated Cancer Progression
Schematic representation of events leading to carcinogenesis of CAC. Red represents gene loss, whereas gray represents genes and cytokines activated during cancer progression.

induces DNA methyltransferase favoring silencing of tumor suppressor genes involved in CAC formation (Hahn et al., 2008).

Furthermore, multiple cytokines activate pro-tumorigenic pathways leading to aberrant inflammation, tumor growth, tumor survival, and angiogenesis. Importantly, it has been shown that reducing inflammation protects from inflammation-associated colon tumorigenesis. In this regard, genetic ablation of p38α in intestinal tissue (involved in the regulation of cytokine production and inflammatory responses) ameliorates tumor formation and protects against inflammation-associated colon tumorigenesis (Youssif et al., 2018).

As mentioned, cytokines have an important role in regulating intestinal inflammation and homeostasis (Table 1). Upon damage, cytokines play an important role in protecting and supporting the regeneration of the intestinal epithelium (Karin and Clevers, 2016). However, aberrant cytokine production and regeneration have been shown to play an essential role in IBD, CAC, and CRC pathogenesis (Neurath, 2014). Pro-tumorigenic cytokines, such as the tumor necrosis factor (TNF), IL-6 and IL-1 can activate receptors present in the intestinal mucosa, triggering oncogenic signaling pathways; and cytokines such as IL-10 and TGF-β have been demonstrated to have a tumor-suppressing role (Grivennikov et al., 2009).

Among the key cytokines involved in CRC, IL-6 has been shown to be highly expressed in murine and human CRC (Brandt et al., 2018; Grivennikov et al., 2009), and it has been reported to act as a crucial mediator controlling cancer initiation, progression, and angiogenesis (West et al., 2015). IL-6 signals from the *lamina propria* to activate the JAK/STAT3 and YAP signaling pathways, which stimulate cell proliferation, survival, and tissue regeneration (Grivennikov et al., 2009; Karin and Clevers, 2016; Taniguchi et al., 2015).

Upon STAT3 axis activation by IL-6, cell stemness and malignancy is promoted through transcriptional and post-translational upregulation of FOSL1 and FRA1 (Wang et al., 2019). Moreover, IL-6 has been shown to trigger proliferation of premalignant enterocytes (in both CAC and Wnt-dependent CRC development) (Fenton et al., 2006; Grivennikov et al., 2009). Other recent studies showed that *in vitro* treatment with IL-6 enhanced cancer cell migration, invasiveness, and proliferation, contributing to tumorigenesis (Zhang et al., 2018).

Additionally, IL-6 has been described to be upregulated synergistically in CRC triggered by inactivation of mTORC1, DNA damage, and high CIN (Brandt et al., 2018). Importantly, IL-6 signal transduction inhibition through IL-6R-specific antibodies or gp130-Fc fusion proteins impairs tumor growth (Neurath, 2014), and diminishes myeloid-derived suppressor cells, which contributes to tumor formation (Li et al., 2019). Furthermore, blocking inflammation and IL-6 reduces hyper-proliferation, restores intestinal

Cytokine	Cellular Source	Function
TNF	Hematopoietic cells	Activation of AP-1, MAPK, and NF-κB, promotes survival and growth
IL-6	Macrophages, T cells, cancer-associated fibroblasts, mesenchymal stem cells, intestinal epithelial cells	Activation of STAT3, ERK, Akt; promotes survival, growth, T cell recruitment; prevents apoptosis
TGF-β	Cancer-associated macrophages	Anti-tumorigenic, pro-apoptotic
IL-1β	Macrophages, neutrophils	Activation of NF-κB and MAPK, promotes survival, growth, and T cell activation
IL-11	Cancer-associated fibroblasts	Activation of STAT3, STAT1, and ERK, promotes survival and growth
IL-23	Macrophages, dendritic cells	Activation of STAT3, promotes T cell differentiation
IL-22	Innate lymphoid cells, T cells	Activation of STAT3, promotes survival and mucosal integrity
IL-17A	Th17 cells, CD4+ T cells, δγ T cells, innate lymphoid cells, cancer-associated fibroblasts	Activation of NF-κβ and MAPK, promotes survival, regulates T cells recruitment
IL-18	Macrophages, intestinal epithelial cells	Anti-inflammatory, protects against CAC
IL-8	CD44 + cancer stem-like cells	Promotes growth and metastasis
IL-15	Transformed cells	Promotes activation of NK cells and CD8+ T cells, stimulates IL-10 production
IL-10	T cells, monocytes	Anti-inflammatory

Table 1. Cytokines Involved in the Development of CRC

homeostasis, and prevents from chronic inflammation and tumor formation in models of CRC triggered by mTORC1 inactivation and suppresses CAC tumorigenesis in an AOM/DSS background (Brandt et al., 2018; Li et al., 2019).

TNF-α activates transcription factors such as AP-1, NF-κB, and MAPK. Importantly, TNF is secreted during the initial inflammatory response and propagates inflammation by inducing the production of other cytokines and chemokines. Additionally, TNF increases endothelial permeability allowing the recruitment of activated leukocytes (Terzić et al., 2010). TNF expression is induced during colon tumorigenesis, and its blockage attenuates CAC formation in mice (Popivanova et al., 2008); thus, blocking TNF is a standard treatment for IBD patients (Danese and Fiocchi, 2013). IL-1 is also known to be upregulated in patients with CAC regulating survival and growth of cancerous cells through the NF-κB and MAPK signaling pathways (Garlanda et al., 2007). Blocking IL-1β in mice using soluble IL-1 receptor agonist reduces tumor infiltration, IL-6 expression, and tumor formation (Wang et al., 2014).

MOUSE MODELS OF CRC

CRC development involves genetic and epigenetic aberrations and multiple molecular pathways. Owing to its high grade of heterogeneity many mouse models have been developed to study CRC pathogenesis and development and, furthermore, potential therapeutic approaches (Tables 2 and 3). Genetically engineered mouse models are widely used, and some involve mutations in proteins that act on the Wnt signaling pathway. For example, the Apc$^{Min/+}$ model presents an autosomal dominant mutation in the codon 850 of the *Apc* gene, leading to the spontaneous LOH of the second Apc allele and, therefore, to the development of intestinal polyps at early stages due to Wnt signaling overactivation (Moser et al., 1990).

Moreover, chemical compounds can also be used to trigger CRC, for example, by combining azoxy-methane (AOM, methyl-methylimino-oxidoazanium) and dextran sulfate sodium (DSS). In this regard,

Model	Strategy	Primary Location	Time to Develop	Number of Tumors	Invasiveness
ApcMin^{+/-}	Germline truncating mutation, model of FAP	Small intestine	100 days	>200	Non-invasive adenoma
Apc[468]	Germline truncating mutation	Small intestine	5 weeks	5–15	Non-invasive adenoma
Apc[15lox]/FabCre	Conditional deletion	Small intestine and colon	5–7 months	10–20	Non-invasive adenoma
Ctnnb[3lox]/FabCre	Activating deletion of exon 3	Small intestine	4 weeks	>200	Non-invasive adenoma
Msh2^{-/-}	Germline deletion, model of HNPCC	Small intestine	6 months	2–6	Adenoma and carcinoma
PIK3ca*/FabCre	Activating mutation	Proximal colon	40 days	N/A	Invasive carcinoma
PTEN[f/f] VillinCreERT2	Conditional deletion	Small intestine	12 months	N/A	Adenoma and carcinoma
PTEN[f/f],KRAS[F12V/+] VillinCreERT2	Conditional deletion of PTEN and activation of KRAS	Small intestine	1 year	N/A	41% of mice show metastasis
TgfbrIl[f/f],KRAS[F12V/+] VillinCreERT2	Conditional deletion of TGF-β and KRAS activation	Small intestine and colon	22 weeks	3–17	15% of mice develop metastasis
Ink4a/Arf^{-/-}Kras[G12V/+] VillinCreERT2	Conditional deletion of INK4a/Afr and KRAS activation KRAS	Proximal colon	12 weeks	1–2	62% of mice develop metastasis

Table 2. Commonly Used CRC Mouse Models for the Study of Sporadic CRC

AOM is a colon-specific carcinogen that triggers tumor formation by inducing alkylation of DNA facilitating base mismatch (Neufert et al., 2007), and DSS is a pro-inflammatory agent that upon administration in drinking water triggers severe colitis associated with colon ulceration and bleeding (Neufert et al., 2007). Although mouse models represent good approaches to study mechanisms of CRC development, it is important to mention that they do not fully recapitulate the heterogeneity of human disease.

DISCUSSION

CRC is a very heterogenic disease, in which many factors act together to promote tumorigenesis. Although *APC* mutations are well established to predispose tumor formation in both genetic and sporadic cases, tumors require further accumulation of mutations and aberrations, and hence genomic instability, for tumor initiation and progression. These somatic events are manly arising from environmental factors most likely implicating poor diet, dysbiosis, and diverse inflammatory cues. Yet, cells at the origin of CRC remain poorly understood. Cell plasticity has recently been suggested to cause a bidirectional conversion between ISCs and differentiated cells triggered by an inflammatory stroma. Lgr5 ISCs has been proposed to play a critical role in CRC (Schepers et al., 2012), whereas recent findings suggest that intestinal tumorigenesis is initiated by dedifferentiation and acquisition of stem-cell-

Model	Strategy	Primary Location	Develop Tumors	Number of Tumors	Invasiveness
AOM/DSS	Chemically induced	Colon	100 days	5–20	Aberrant crypt foci and adenomas
Il10^{-/-}	Germline deletion	Colon and rectum	6 months	N/A	Adenocarcinoma
Muc2-/-	Germline deletion	Small intestine and colon	2 years	1–2	Adenomas and invasive adenocarcinoma
Rag2^{-/-}, Tgfb1^{-/-}	Germline deletion	Colon and cecum	3 months	>30	Adenocarcinoma

Table 3. Commonly Used CRC Mouse Models for the Study of CAC

like properties caused by NF-κB signaling (Schwitalla et al., 2013). Deep understanding of molecular and cellular mechanisms and genetic phenotype may be of primordial importance for the development of more efficient therapies.

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AUTHOR CONTRIBUTIONS

T.P.G, M.B wrote and revised the manuscript in equal contribution. T.P.G. performed the graphical abstract and figures. M.B performed the tables. N.D contribute to the review and editing of the manuscript and secured funding.

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