Molecular and Cellular Mechanisms of Carcinogenesis in the Large Bowel

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Contents

Abstract

Colorectal cancer is one of the most intensively studied cancers with well-documented precursor lesions. The acquisition of genomic instability plays a central role in its development. In the majority of cases, tumor growth results from different combinations of sporadic genetic events and epigenetic alterations, resulting in increased cell proliferation and decreased cell death. Three main pathways have been identified: chromosomal instability (CIN)

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pathway, microsatellite instability (MSI) pathway, and CpG island hypermethylation phenotype (CIMP) pathway. Within these pathways, somatic *BRAF* and/or *KRAS* mutations have been identified as major players. Up to 5% of colorectal cancers develop in the setting of inherited syndromes, such as Lynch syndrome, familial adenomatous polyposis, *MUTYH*-associated polyposis, and certain hamartomatous polyposis conditions, including Peutz-Jeghers syndrome and juvenile polyposis syndrome. In this chapter, we describe the above-mentioned pathways and syndromes in detail, referring to different molecular events and different precursor lesions. In the last part, we address possible future perspectives in colorectal carcinogenesis.

4.1 Introduction

Colorectal cancer (CRC) ranks the third most frequent cancer in men (after lung and prostate cancer) and second in women (after breast cancer), representing approximately 9.7% of all new cancer cases diagnosed worldwide [1, 2]. In 2012, an estimated 746.000 men and 614.000 women were diagnosed with CRC, and 694.0000 died of the disease [1, 2]. In the last decade (2001–2010), the global incidence rate decreased by approximately 3% per year [3].

On the molecular level, CRC is one of the most intensively studied cancers. The existence of well-documented precursor lesions indicates multistep cancer development. In fact, this type of cancer represents a very heterogeneous disease regarding the clinical presentation, likelihood of cure, pattern of extension, and response to treatment [4]. The acquisition of genomic instability plays a central role in its development. In the majority of cases, CRC results from different combinations of sporadic genetic events and epigenetic alterations, resulting in increased cell proliferation and decreased cell death [5]. Kindred and twin studies, also studies based on family clusters, estimated that approximately 30% of all CRC cases are inherited [6, 7].

In the last decade, a growing body of scientific evidence demonstrated that different CRC subtypes can be separated based upon combinations of different genetic markers. Three major signaling pathways have been recognized, all characterized by specific precursor lesions, mechanisms of carcinogenesis, and natural history: the chromosomal instability (CIN) pathway, the microsatellite instability (MSI) pathway, and the CpG island hypermethylation phenotype (CIMP) pathway [5, 8] (Fig. 4.1). Within these pathways, somatic *BRAF* and/or *KRAS* mutations have been identified as major players [5]. Up to 5% of CRCs develop in the setting of inherited syndromes like Lynch syndrome (LS), familial adenomatous polyposis (FAP), *MUTYH*-associated polyposis (MAP), and certain hamartomatous polyposis conditions [9].

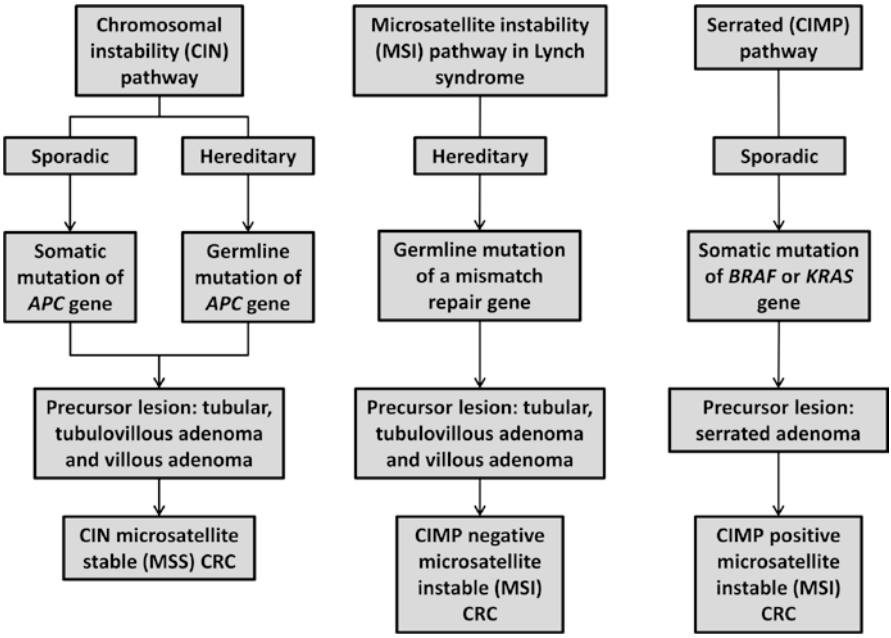

Fig. 4.1 Three major carcinogenic pathways have been identified in colorectal cancer (*CRC*): chromosomal instability (*CIN*), microsatellite instability (*MSI*), and CpG island methylator phenotype (*CIMP*)

4.2 Molecular Classification of Colorectal Cancer

In this chapter, we will describe the three major pathways responsible for CRC: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island hypermethylation phenotype (CIMP). We will also refer to the MAP and to hamartomatous polyposis syndrome, such as Peutz-Jeghers syndrome (PJS) and juvenile polyposis syndrome (JPS), and will finally address possible future perspectives.

4.2.1 The Chromosomal Instability (CIN) Pathway

The CIN pathway, also called the "traditional pathway," is characterized by imbalance in chromosomal number (aneuploidy), subsequent loss of heterozygosity (LOH) of genes, and subchromosomal genetic amplifications [10]. The time of tumor development via this pathway is approximately 10–15 years. The initial lesion in this pathway is the dysplastic aberrant crypt focus (ACF) [11]. It is a microscopic mucosal lesion that develops into conventional adenomas, i.e., tubular

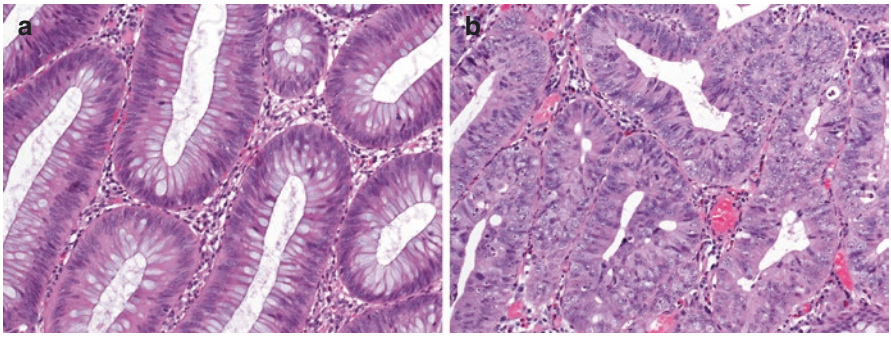

Fig. 4.2 Tubular colorectal adenoma with low-grade dysplasia, characterized by well-formed glands and pseudostratified, polarized, hyperchromatic nuclei (**a**). High-grade dysplasia characterized by increased architectural complexity and more severe atypia with loss of nuclear polarity (**b**)

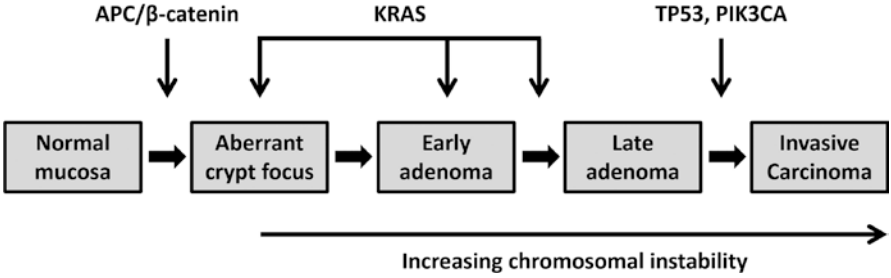

Fig. 4.3 Multistep genetic model of colorectal carcinogenesis (adenoma-carcinoma sequence): chromosomal instability is observed in benign adenomas and increases in conjunction with tumor progression (from [12], S. Karger AG, with permission)

adenomas, tubulovillous, and villous adenomas (Fig. 4.2), which are the macroscopically discernable precursor lesions of sporadic CRCs arising via this pathway [12]. It is of note that traditional adenomas are also considered to be the precursor lesions in the hereditary cancers, namely, LS and FAP [12, 13].

Already in 1990, Fearon and Vogelstein [14] proposed a multistep model of sequential genetic alterations, responsible for adenoma and ultimately carcinoma formation within the colorectum (Fig. 4.3). Mutation in the adenomatous polyposis coli (*APC*) tumor-suppressor gene located on chromosome 5q21 has been identified as the first step of this model [15]. Both copies of the *APC* gene must be functionally inactivated for adenomas to develop. Specifically, *APC* mutation interferes with phosphorylation of β-catenin, a component of the *Wnt* signaling pathway that regulates apoptosis, growth, and differentiation. Consequently, β-catenin is not ubiquitinated and destroyed by the proteasome. It accumulates in the cytosol and is translocated to the nucleus, where it interacts with T-cell factor (TCF)/lymphoid enhancer factor (LEF), converting these effectors into transcriptional activators [16]. Activation of the *Wnt* pathway is present in up to 80% of adenomas [11].

The second molecular event is an activating mutation of Kirsten-rat sarcoma 2 viral oncogene (*KRAS*), which is, however, not unique for this pathway. This

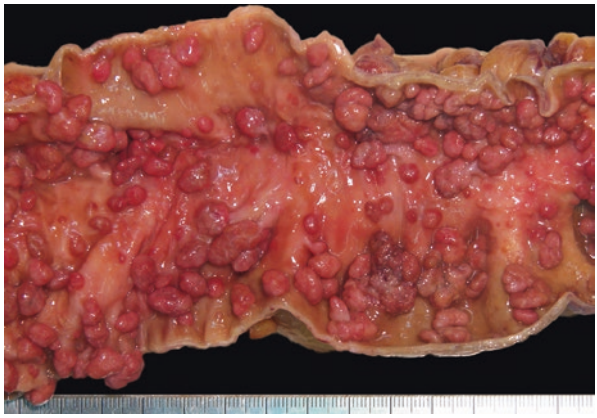

Fig. 4.4 Gross presentation of familial adenomatous polyposis (FAP): the colectomy specimen shows numerous adenomatous polyps

mutation is found in approximately 45% of CRCs and constitutively activates the MAPK signaling pathway [17]. The genomic change in adenoma-carcinoma sequence also includes LOH of chromosome 18q, which is present in up to 60% of tumors [18]. Many important tumor-suppressor genes are located at 18q21.1—*DCC*, *SMAD2*, and *SMAD4*, the latter being involved in TGF-β signaling, responsible for regulation of growth and apoptosis. Mutational inactivation of the tumor-suppressor *TP53* (17p13) occurs as a late event (at the transition from high-grade adenoma to invasive cancer) in up to 80% of CRC [17]. Mutational activation of phosphatidylinositol-4, 5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) occurs also in the late phase, but in a small number of cases [10].

Recognition of the central role of *APC* mutations in tumorigenesis has improved our understanding of FAP, the second most common inherited CRC syndrome. APC-associated polyposis conditions also include attenuated FAP, Gardner syndrome (FAP with epidermoid cysts, osteomas, dental anomalies, and/or desmoid tumors), and Turcot syndrome (colonic polyps with central nervous system tumors) [9, 19]. FAP is characterized by the development of hundreds to thousands of conventional adenomas beginning in early adolescence (Fig. 4.4). The disease inevitably leads to CRC, thereby prompting prophylactic colectomy. This syndrome accounts for only <1% of all CRCs. The neoplastic polyps are distributed among the colorectum and can also be observed in the stomach and small bowel, in particular the duodenum. Attenuated FAP is a less severe form, characterized by <100 colonic adenomatous polyps with tendency for proximal location. In individuals with attenuated FAP, adenoma and CRC development is delayed by 15 years when compared to classic FAP [20].

4.2.2 Microsatellite Instability (MSI) Pathway and Lynch Syndrome (LS)

Errors that occur during DNA replication are corrected by the mismatch repair (MMR) system, which includes the following proteins: MLH1, PMS2, MSH2, and MSH6. This system is necessary for maintaining genomic stability. During mismatch repair, the MMR proteins form heterodimers, that is, MLH1 builds a complex with

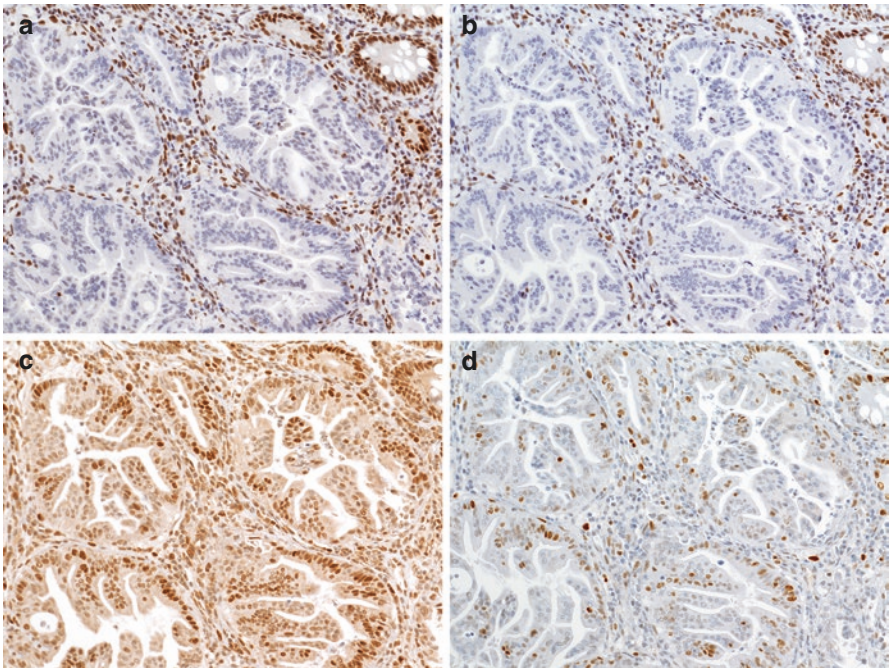

Fig. 4.5 Mismatch repair (MMR) protein expression in a cancer with high-level microsatellite instability (MSI-H): MLH1 (**a**) and PMS2 (**b**) staining is lost in the tumor cell nuclei, while the expression of MSH2 (**c**) and MSH6 (**d**) is retained. Nonneoplastic stromal and inflammatory cells serve as internal positive control (serial sections)

PMS2, and MSH2 builds another with MSH6 [21, 22]. It is well known that the MLH1 and MSH2 proteins are the dominant components of their heterodimers. In consequence, mutations in *MLH1* or *MSH2* gene lead to proteolytic degradation of the corresponding dimer and subsequent loss of both, the main and the auxiliary partner proteins (Fig. 4.5) [23]. If a mutation occurs in one of the auxiliary genes, i.e., *PMS2* or *MSH6*, this results in a loss of the respective PMS2 or MSH6 protein, but does not cause secondary loss of the dominant protein, that is, MLH1 or MSH2 [3].

When the MMR system does not function properly, the cells accumulate genetic errors. These may happen also in so-called microsatellites, that is, repetitive segments of DNA (two to five nucleotides in length), which are found scattered throughout the genome in the noncoding regions between genes or within genes [24]. MSI is defined as a change of any length of these repeating units, due to deletion or insertion [25].

For MSI testing, different panels of microsatellite markers have been used. The first consensus of the National Cancer Institute (NCI) recommended the use of a panel of five markers for MSI testing [26]. This included two mononucleotide repeats (BAT-25 and BAT-26) and three dinucleotide repeats (D5S346, D2S123, and D17S250) [27]. Other panels are solely based upon mononucleotide repeat markers, which can be amplified and analyzed in a single assay [28, 29]. Tumors with instability in two or more of the five markers qualify for high-level MSI (MSI-H; Fig. 4.6), whereas those with instability at one repeat qualify for low-level MSI

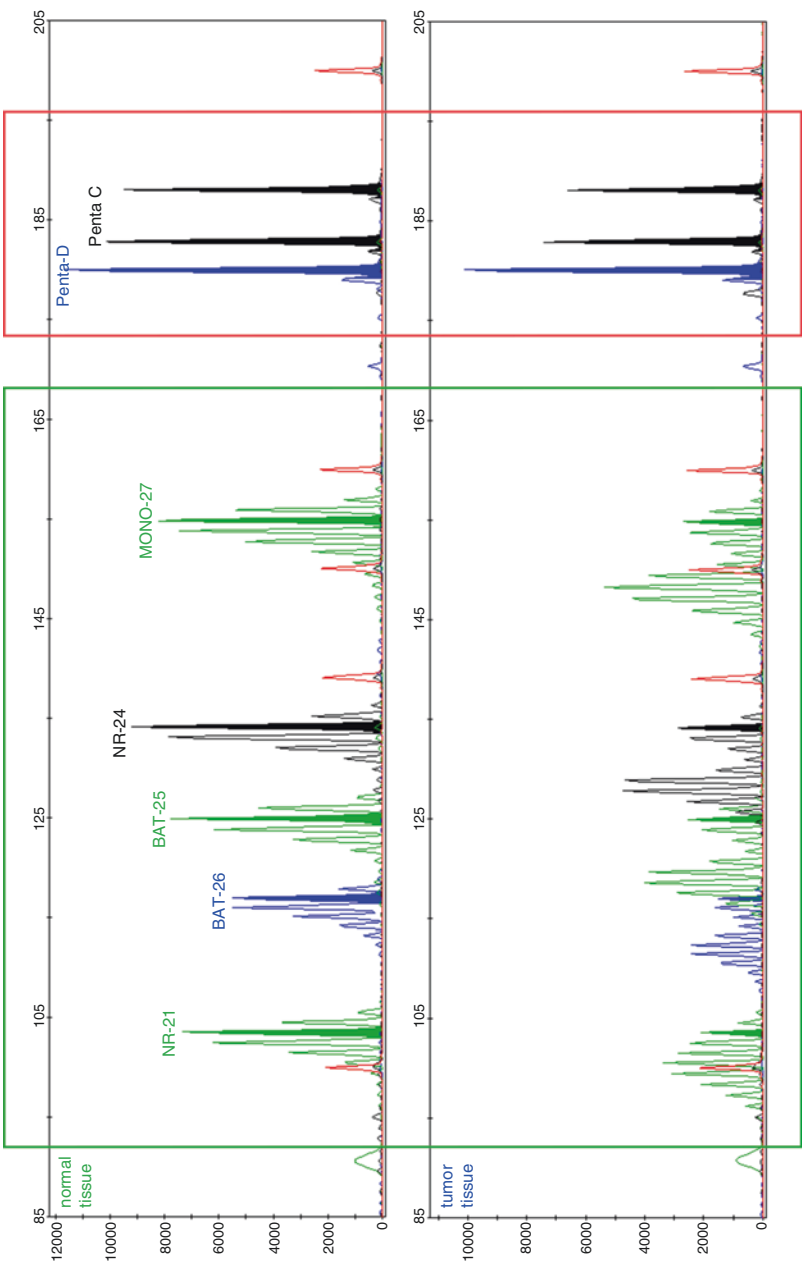

Fig. 4.6 Example of cancer with high-level microsatellite instability (MSI-H): The MSI profile is assessed by a panel of five monomorphic mononucleotide repeats. Instability for all markers is observed, as shown by additional alleles (allelic shifts). Two polymorphic pentanucleotide repeats (Penta C and Penta D) are included for sample identification

(MSI-L). When all markers are stable, the lesion is called microsatellite stable (MSS) [4, 28].

Approximately 15% of CRCs are genetically instable due to MSI [30]. The majority of these tumors (80%) are sporadic and arise due to hypermethylation of the *MLH1* gene promoter [31]. Other 20% of tumors are inherited, that is, caused by germ line mutation in one of the MMR genes and associated with LS [32]. This syndrome follows an autosomal dominant trait of inheritance and accounts for 2–4% of all CRCs [33, 34]. Specifically, mutations in *MLH1* and *MSH2* account for most cases (approximately 40% each), while mutations in *MSH6* and *PMS2* account for only 10% and 5%, respectively [33, 35].

CRCs in LS usually occur at early age (approximately 45 years) and are right-sided (approximately 70% proximal to the splenic flexure) [33]. In addition, they may be multifocal with syn- and/or metachronous tumor development, and there is also a higher risk for extracolonic tumors [36]. These mainly include endometrial, ovary, and gastric tumors [9].

The lifetime risk of cancer in LS is depending on sex and the mutated MMR gene [37–44]. In patients with *MLH1* or *MSH2* mutation, the risk of CRC has been calculated 27–74% for males and 22–53% for females, respectively, with mean age at diagnosis varying from 27 to 46 years (69 years for sporadic cancers). The risk of endometrium cancer is 14–54% [45]. When *MSH6* is mutated, the CRC risk appears to be lower (18%), while the endometrium cancer risk is not changed. Smaller studies reported a lower *PMS2* mutation penetrance for CRC and endometrium cancer as compared with *MLH1* and *MSH2* mutation carriers and similar or even lower risks as compared with *MSH6* mutation carriers [46]. A large European cohort recently reported a cumulative risk of CRC of 19% for males and 11% for females, while the risk of endometrium cancer was 12%. In this cohort, the mean age at diagnosis for both CRC and endometrium cancer was higher as compared with *MLH1* or *MSH2* mutation carriers. When compared with *MSH6*, the mean age at diagnosis of CRC was lower, and the mean age at diagnosis of endometrium cancer was similar [46].

Several tools are available to assist the clinical diagnosis of LS, including analyses of family history, tumor testing, mutation prediction models, and genetic testing. The Amsterdam criteria were created first in 1990 and then reestablished in 1999 as Amsterdam criteria II defining clinical criteria needed for the diagnosis of HNPCC [45, 47–51]. These criteria include individual patient and family history of colonic and extracolonic tumors. They are listed in Table 4.1.

The revised Bethesda guidelines are a third set of clinicopathologic criteria developed to identify individuals that should be investigated for LS by evaluation of MSI and/or immunohistochemical (IHC) testing (Table 4.2) [52].

Table 4.1 Amsterdam criteria I and II for the diagnosis of Lynch syndrome [45, 47–51]

Amsterdam criteria I
1. Three or more relatives with histologically verified CRC, one of which is a first-degree relative of the other two
2. Two or more generations should be affected
3. One or more patients with CRC should be diagnosed before the age of 50 years
4. Familial adenomatous polyposis (FAP) should be excluded
Amsterdam criteria II
1. Three or more relatives with histologically verified Lynch syndrome-associated cancer (CRC, cancer of the endometrium, small bowel, ureter, or renal pelvis), one of which is a first-degree relative of the other two
2. Two or more generations should be affected
3. One or more cancer patients should be diagnosed before the age of 50 years
4. Familial adenomatous polyposis (FAP) should be excluded

Table 4.2 The revised Bethesda Guidelines [45, 49–52]. Colorectal cancers (CRCs) should be tested for microsatellite instability (MSI) in the following settings:

1. CRC diagnosed in a patient who is less than 50 years of age
2. Presence of synchronous or metachronous CRC or other Lynch syndrome-associated tumor[a], regardless of age
3. CRC with MSI-H histology diagnosed in a patient who is less than 60 years of age
4. Patient with CRC and CRC or Lynch syndrome-associated tumor[a] diagnosed in at least one first-degree relative less than 50 years of age
5. Patient with CRC and CRC or Lynch syndrome-associated tumor[a] diagnosed in two first-degree or second-degree relatives, regardless of age

[a]Lynch syndrome-associated tumors: cancers of the colorectum, endometrium, stomach, ovary, pancreas, biliary tract, small bowel, ureter, and renal pelvis; brain tumors (usually glioblastoma as seen in Turcot syndrome); sebaceous gland adenomas; and keratoacanthomas (in Muir-Torre syndrome)

Adenomas and CRCs in LS arise earlier and at more proximal location when compared to sporadic neoplasm. The rate of adenoma development is similar to the rate of adenoma development in the sporadic setting, but progression to cancer occurs at increased rate. This is in contrast to FAP, which has an increased rate of adenoma formation, while progression to cancer is believed to occur at a similar rate to that of sporadic adenomas. In LS, the germ line inactivation of one of the mismatch repair genes, coupled with somatic inactivation of the remaining allele, increases the mutation rate and, subsequently, the rate of progression from adenoma to cancer (Fig. 4.7) [12, 53].

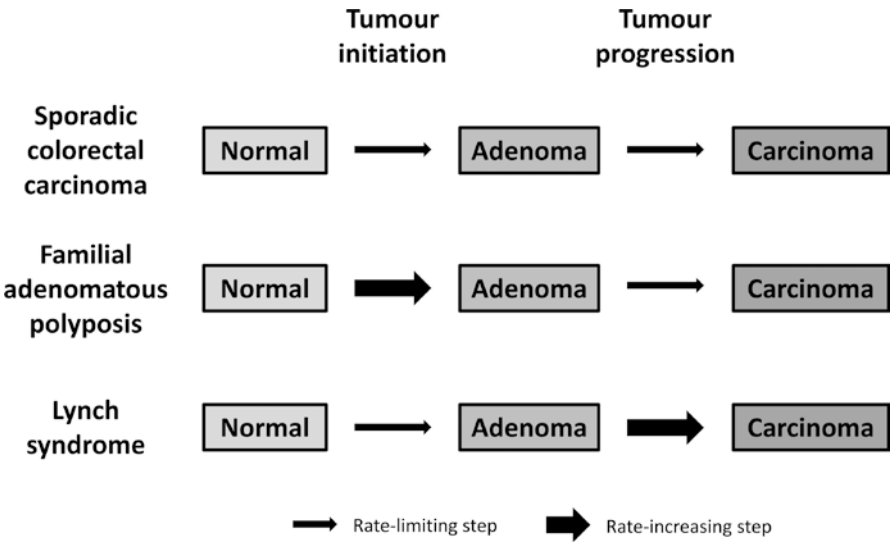

Fig. 4.7 Relative effects of germ line mutations on the rate of tumor initiation and progression: In sporadic tumors, adenoma formation and cancer development are rate-limiting steps. In familial adenomatous polyposis, adenoma formation occurs at an increased rate, while adenomas progress to cancer at a rate similar to the sporadic setting. The mutation rate within adenomatous polyps and, subsequently, the rate of progression from adenoma to cancer are increased in Lynch syndrome (from [12], S. Karger AG, with permission)

4.2.3 CpG Island Methylator Phenotype (CIMP) and Serrated Pathway

CpG dinucleotides (cytosine nucleotide followed by a guanine nucleotide) are uncommon in the human genome. However, in the promoter region of about half of all genes, clusters of these nucleotides, called CpG islands, are found [54]. Aberrant (hyper)methylation of CpG-rich promoters leads to epigenetic silencing of tumor-suppressor genes and ultimately cancer. The methylation status of the tumor can be assessed according to the degree of methylation as CIMP high, CIMP low, or CIMP negative [55]. However, molecular analysis of CIMP and classification of methylation level appear to be poorly standardized. Hence, up to date, no precise definition of CIMP and no consensus recommendation are available [3].

Sporadic MSI-H CRCs occur in patients without germ line mutation in a MMR gene. These tumors occur preferably in the right colon. They are diagnosed more commonly in women, often at advanced age [56, 57]. These cancers develop from serrated precursor lesions [31] through the CIMP or "serrated pathway" (Fig. 4.8), characterized by *BRAF* mutation (characteristically V600E) and hypermethylation in CpG-rich gene promoters, which leads to silencing of distinct tumor-suppressor genes, including the MMR gene *MLH1*, as well as *p16*, *MGMT*, and *IGFB7* [58–62].

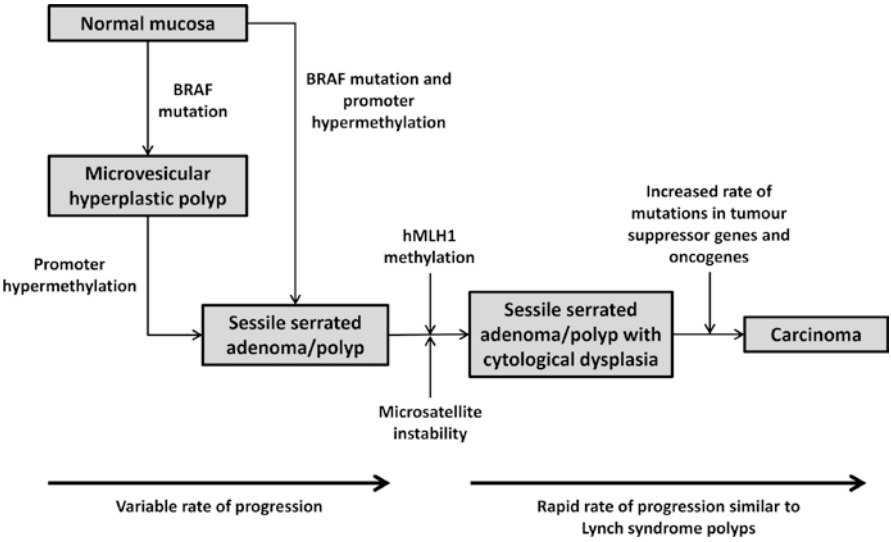

Fig. 4.8 Colorectal carcinogenesis according to the "serrated (CIMP) pathway": sporadic colorectal adenocarcinomas with high-level microsatellite instability (MSI-H) develop from serrated precursor lesions due to promoter hypermethylation of the *MLH1* gene (from [12], S. Karger AG, with permission)

Sessile serrated adenomas/polyps (SSA/P) are considered to be the main precursor lesions of the serrated pathway. They account for approximately 5–25% of all serrated lesions occurring in the colorectum [13, 63, 64]. They may arise from large microvesicular hyperplastic polyps or develop de novo from normal colonic mucosa. Uncomplicated SSA/Ps do not show dysplasia. Dysplasia may, however, occur during neoplastic progression (Fig. 4.9). There appears to be a histological continuum from non-dysplastic ACF to microvesicular hyperplastic polyps to SSA/P to SSA/P with cytological dysplasia and ultimately to invasive ("serrated") adenocarcinoma [12].

Serrated lesions can also occur in familial setting. Serrated polyposis syndrome is a rare condition characterized by multiple and/or large serrated polyps of the colon. Guarinos et al. [65] identified *BRAF* mutations in 63% and *KRAS* mutations in 10% of lesions occurring in this syndrome; 43% of the lesions were CIMP high. A single per patient analysis showed that all patients had *BRAF* or *KRAS* mutation in more than 25% of the polyps, and 84.8% of patients had a mutation in *BRAF* or *KRAS* in more than 50% of their polyps [65]. Germ line loss-of-function mutations in oncogene-induced senescence pathways may play an additional role in the disease [66].

Traditional serrated adenomas (TSAs) are much less common than the other serrated lesions, accounting for approximately 1% of colorectal polyps (Fig. 4.10). The majority of lesions are detected in the distal colon [12]. TSAs may originate from preexisting non-dysplastic serrated polyps, including hyperplastic polyps and SSA/Ps.

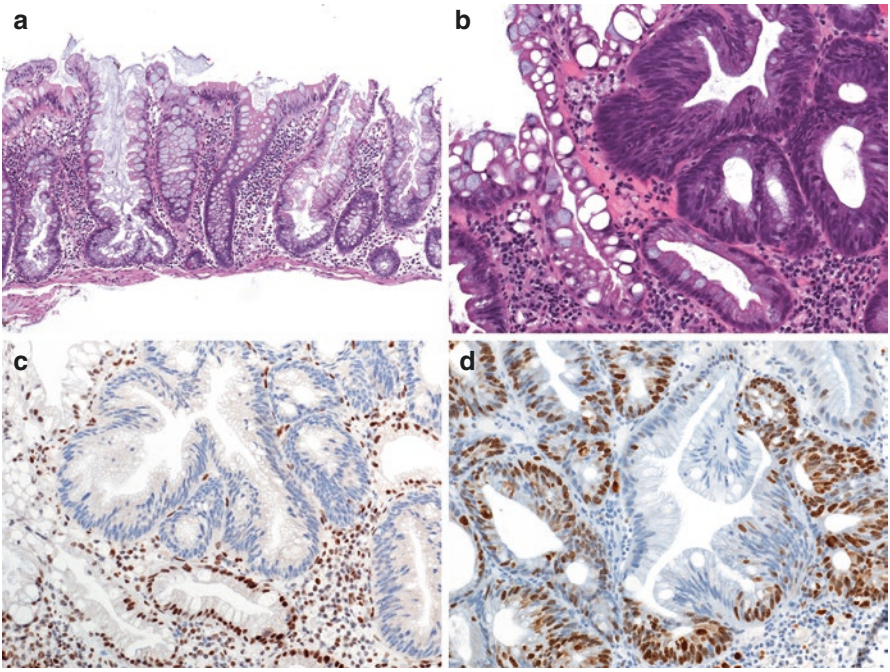

Fig. 4.9 Sessile serrated adenoma/polyp (SSA/P) with increased serration of non-dysplastic crypts, with T-shaped ("anchor") crypts and mature goblet cells at the crypt bases (**a**). Cytological dysplasia is not present in uncomplicated SSA/P, but develops with progression toward carcinoma (**b**), often in conjunction with promoter hypermethylation of the *MLH1* gene, as illustrated by loss of nuclear MLH1 expression (**c**). The proliferation rate (MIB-1) is markedly increased in the dysplastic glands (**d**)

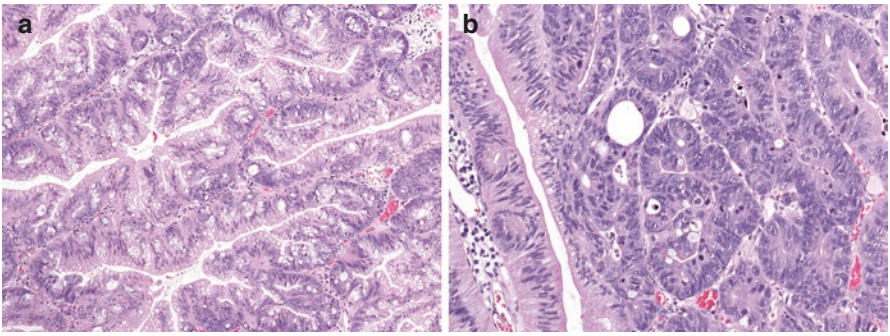

Fig. 4.10 Traditional serrated adenoma (TSA) with slit-like serration, cytoplasmic eosinophilia, and proliferative "ectopic crypts" (**a**). In high-grade dysplasia, marked architectural complexity and nuclear atypia with increased nuclear/cytoplasmic ratio are observed (**b**)

On the molecular level, these lesions are characterized by *BRAF* mutations, giving rise to *BRAF*-mutated MSS CRCs [67, 68]. TSAs may alternatively develop de novo. These lesions mainly show mutations in the *KRAS* gene. Malignant progression occurs via *TP53* mutation and *Wnt* pathway activation regardless of mutation status [67–69].

4.2.4 MUTYH-Associated Polyposis (MAP)

MAP is a hereditary condition caused by biallelic germ line mutations in *MUTYH* gene and has an autosomal recessive pattern of inheritance [70]. It is characterized by the development of multiple neoplastic polyps in the colorectum and increased risk of CRC [9]. The colonic phenotype of MAP mimics FAP—however, in addition to multiple adenomatous polyps, hyperplastic polyps and SSA/Ps can also be found [71].

The *MUTYH* gene product is involved in the base-excision repair pathway and protects against oxidative DNA damage. Individuals with >10 colorectal adenomas who do not have mutation in *APC* should undergo genetic testing for MAP [9].

4.2.5 Hamartomatous Polyposis Conditions

Hamartomatous polyposis conditions include PJS, JPS, hereditary mixed polyposis syndrome, Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and Cronkhite-Canada syndrome [72]. This group of disorders is characterized by the development of multiple benign-appearing polyps in the gastrointestinal tract. Affected individuals bear an increased risk of cancer, not only in the gastrointestinal tract but also in other organs [9]. Carcinogenesis, that is, progression of the hamartomatous polyps to cancer or cancer development de novo, is still largely unclear, suggesting different pathways from adenomatous polyposis. In this chapter, we will concentrate on the two most common conditions, that is, PJS and JPS. They can both be sporadic or familial, in the hereditary setting having an autosomal dominant pattern of inheritance.

In PJS the key clinical features are hyperpigmentation (melanosis) of the lips, mouth, and oral mucosa and polyposis of the small intestine. Affected individuals harbor a mutation in the *STK11* gene [73]. Lifetime cancer risk is as high as 81–93%, with 50% risk for breast cancer and 39% risk for colon cancer [74].

JPS is caused by germ line mutations in either *MADH4* (*SMAD4, DPC4)* or *BMPR1A*, which can be found in 18.2% or 20.8% of affected individuals, respectively [75]. The condition is characterized by multiple hamartomatous polyps, most commonly arising in the colon but rarely also in the stomach, duodenum, and small bowel. For both sporadic and familial JPS, mean age of CRC diagnosis is 37 years [76]. Lifetime cancer risk has been estimated 38% for colonic and 21% for upper GI cancers, including the stomach, pancreas, and small bowel [77].

4.3 Future Perspectives

Recent data indicate an even greater complexity of cancer development in the colorectum. Thus, germ line exonuclease domain mutations (EDMs) of *POLE* and *POLD1* have been shown to confer high risk of multiple colorectal adenomas and carcinoma, a condition named polymerase proofreading-associated polyposis (PPAP). Somatic *POLE* EDMs have also been found in sporadic CRCs and endometrial cancers. It is believed that both the germ line and the somatic mutations cause impair polymerase proofreading resulting in "ultramutated," yet microsatellite stable (MSS), tumors [78].

In addition, Guinney et al. [79] reported four "consensus molecular subtypes" (CMS) of colorectal cancer: CMS1 (MSI immune, 14%), hypermutated, microsatellite unstable, and strong immune activation; CMS2 (canonical, 37%), epithelial, marked WNT, and MYC signaling activation; CMS3 (metabolic, 13%), epithelial and evident metabolic dysregulation; and CMS4 (mesenchymal, 23%), prominent transforming growth factor-β activation, stromal invasion, and angiogenesis. It is of note that 13% of samples tested showed mixed features, which could be explained by intratumoral heterogeneity or by a transition phenotype. The significance of this "consensus" publication is, however, currently unclear.

Conclusion

Different molecular and cellular mechanisms of carcinogenesis have been identified in the large bowel. These mainly include CIN, MSI, and CIMP pathways. Familial cancers may arise within FAP, LS, and MAP syndromes. Hamartomatous polyposis syndromes likewise harbor increased cancer risk. Four consensus molecular subtypes (CMS1-CMS4) have been described recently, awaiting validation by other groups.

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