REVIEW – CANCER RESEARCH

Genomic landscape of colorectal carcinogenesis

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Abstract

Purpose The molecular pathogenesis of solid tumour was first assessed in colorectal cancer (CRC). To date, ≤ 100 genes with somatic alterations have been found to inter-connectively promote neoplastic transformation through specific pathways. The process of colorectal carcinogenesis via genome landscape is reviewed on the basis of an adenoma-to-carcinoma sequence, as shown by serial histological and epidemiological observations.

Methods The relevant literatures from PubMed (1980–2021) have been reviewed for this article.

Results The major routes of CRC development, chromosomal instability (CIN), microsatellite instability (MSI), and the serrated route either via CIN or MSI, proceed through the respective molecular pathway of colorectal carcinogenesis. Particular aspects of CRC carcinogenesis can also be determined by evaluating familial CRCs (FCRC) and genotype–phenotype correlations. Specific causative gene alterations still leave to be identified in several FCRCs. Otherwise, recently verified FCRC can be particularly notable, for example, *EPCAM*-associated Lynch syndrome, polymerase proofreading-associated polyposis, *RNF43*-associated polyposis syndrome or *NTHL1* tumour syndrome, and hereditary mixed polyposis syndrome. The oncogenic landscape is described, including representative pathway genes, the three routes of carcinogenesis, familial CRCs, genotype–phenotype correlations, the identification of causative genes, and consensus molecular subtypes (CMS).

Conclusion Whole genome research using multi-gene panels (MGPs) has facilitated high through-put detection of previously unidentified genes involved in colorectal carcinogenesis. New approaches designed to identify rare variants are recommended to consider their alterations implicated in the molecular pathogenesis.

Keywords Colorectal cancer · Carcinogenesis · Routes · Pathway · Hereditary · Genome · Epigenome

Introduction

Tumour initiation is traditionally regarded as a stepwise accumulation of alterations, leading to an expansion of clones with selective growth advantages, with the fittest clone eventually becoming dominant (Fearon and Vogelstein 1990). This sequential model of colorectal carcinogenesis has been confirmed by serial histological and epidemiological observations (Luebeck and Moolgavkar 2002). The most prevalent molecular alterations, including chromosomal instability (CIN), microsatellite instability (MSI), and somatic mutations involving the TGF-β and Wnt pathways, are synergistically involved in early carcinogenesis, cancer stem cell (CSC) maintenance, and tumour progression (Angius et al. 2021). By contrast, the "Big Bang" model suggests that tumours grow predominantly as a single expanding clone producing numerous mixtures of sub-clones that are not subject to stringent selection, with both public (clonal) and most detectable private (sub-clonal) alterations arising soon after the initial step of tumour progression (Sottoriva et al. 2015).

The gene causing familial adenomatous polyposis (FAP) was initially identified by a deletion in chromosome 5, as determined by linkage analysis of DNA markers from 124 subjects in 13 different FAP families (Bodmer et al. 1987),

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with further analysis showing that this locus was located at chromosome 5q22.2. The classical pathway of colorectal cancer (CRC) progression was hypothesized to involve an "adenoma–carcinoma" sequence, in which the initial molecular alterations lead to the formation of benign tumours, such as adenomas and serrated polyps, followed by additional molecular steps that give rise to more histologically invasive tumours (Fearon and Vogelstein 1990).

This review summarizes the oncogenic pathways associated with colorectal carcinogenesis, including identified genes and their interactions. The oncogenic landscape is described, including representative pathway genes, the three routes of carcinogenesis, familial CRCs, genotype–phenotype correlations, the identification of causative genes, and consensus molecular subtypes (CMS).

Oncogenomic landscape and colorectal cancer

To date, several hundred driver genes have been identified and shown to be altered by intragenic mutations, with other mutations being passengers not associated with selective growth advantages (Martínez-Jiménez et al. 2020). Alternatively, the differentiation between driver and passenger genes may depend simply on the strength of selections associated with given genetic mutations or epigenetic changes (Crouch and Bodmer 2020). Thus, the stronger drivers may be those genes that are more frequently mutated, whereas it may be difficult to determine whether particular genes with relatively few mutations are really "neutral" or "passengers".

DNA methylation accompanied by transcriptional repression facilitates the oncogenic process but does not exclude the possibility of subsequent mutations. DNA methylation has been associated with a wide range of molecular changes, including changes in growth rate, inflammation, and metaplasia, not only genetic changes. Aberrant methylation in a subset of *MLH1*, *MGMT*, and *HIC1* genes can be pathogenic in cancer (Grady and Carethers 2008). Nucleosomal core histones possess specific tails, which can be subjected to different modifications, including acetylation, methylation, ubiquitination, phosphorylation, and sumoylation (https://www.genecards.org/). Histone modifications resulting in gene dysregulation have been identified in various malignancies. These modifications can result in aberrant expression of histone deacetylases, such as HDAC1 and HDAC2; mutations in genes encoding histone acetyltransferases, such as P300, CBP, and PCAF; and/or overexpression of histone methyltransferases such as EZH2 in CRCs (Esteller 2007).

Enteric microbes can also participate in colorectal carcinogenesis and progression through their metabolites and toxins. Approximately 2% of CRCs have been linked to colitis, including colitis induced by *Bacteroides fragilis*

toxins and the adherent-invasive *Escherichia coli* strain NC101 (Mizutani et al. 2020). Another periodontal pathogen, *Fusobacterium nucleatum*, was reported to induce non-colitis-associated CRCs. Secondary bile acids may also be involved in CRC cell proliferation, through β-catenin activation, ERK1/2 activation, the c-Myc target pathway, and the NF-κB signalling pathway (Pai et al. 2004).

Representative pathways and related genes

Molecular signalling of colorectal carcinogenesis can be divided into nine major pathways, called the Wnt/β-catenin, JAK/STAT, RAS/ERK, PI3K/AKT, TGF/SMAD, Notch, Hedgehog, TNF-related apoptosis-inducing ligand (TRAIL), and p53 pathways (Farooqi et al. 2019; Kim et al. 2008). These nine pathways interact closely to form intra- and inter-cellular networks that induce colorectal carcinogenesis (Fig. 1).

Wnt/β-catenin

The canonical Wnt/β-catenin (or Wnt) pathway involves the accumulation of β-catenin in the cytoplasm and its translocation into the nucleus, where it acts as a transcriptional coactivator of transcription factors belonging to the TCF/LEF family (Deitrick and Pruitt 2016). Subsequently, β-catenin
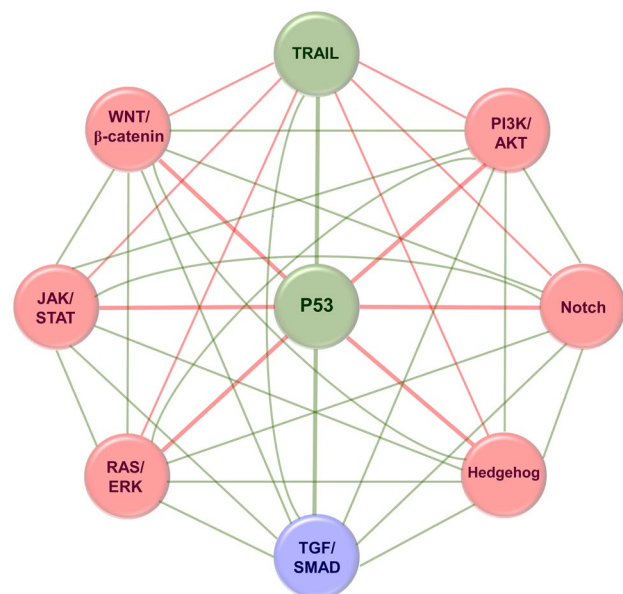

Fig. 1 The nine major pathways associated with colorectal carcinogenesis. The nine representative pathways, drawn as circles, were the tumour suppressor (green), oncogenic (red), and facultative (blue) pathways. Molecular interactions between two pathways were either synergistic (green lines) or suppressive (red lines), as well as being strong (thick lines) or modest (thin lines)

is targeted for ubiquitination and subsequent proteasomal degradation by a degradation complex consisting of axin, adenomatous polyposis coli (APC), casein kinase 1 (CK1), and glycogen synthase kinase-3β (GSK-3β). More than half of CRCs harbour oncogenic mutations in regulatory components of the Wnt pathway, with their deregulation being primary drivers of colorectal carcinogenesis (Farooqi et al. 2019). In particular, *APC* mutations, found in approximately 80% of all CRCs, result in the unrestrained activation of the Wnt pathway (van Neerven et al. 2021). Tumours with microsatellite instability (MSI) frequently harbour mutations in regulatory components of the Wnt pathway, including truncating, but mutually exclusive, mutations in *AXIN2* and *TCF7L2* (Kim et al. 2009). The E3 ubiquitin ligase RNF43, which is associated with serrated polyposis syndrome, was found to regulate Wnt signalling by inducing the degradation of the Wnt receptor Frizzled (Tsukiyama et al. 2020). Additionally, the Wnt-dependent endogenous Rspo2 and Rspo3 chromosomal rearrangements can initiate and maintain colorectal carcinogenesis (Han et al. 2017).

JAK/STAT

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) signalling pathway is involved in colorectal carcinogenesis via immune function and cell growth (Spano et al. 2006). Activated JAKs phosphorylate receptors at specific tyrosine residues, resulting in the recruitment of cytoplasmic monomeric STAT proteins via their SH2 domains (Spano et al. 2006). The SOCS protein belongs to a group of cytokine-inducible genes that have been shown to inhibit STAT signalling by binding to JAKs. STATs participate in oncogenesis by upregulating the expression of genes encoding apoptosis inhibitors (Bcl-x1, Mcl-1), cell cycle regulators (cyclins D1/D2, c-Myc), and inducers of angiogenesis (VEGF) (Buettner et al. 2002).

Ras/ERK

The Ras/ERK signalling pathway is a well-characterized series of kinase cascades involved in cell growth and proliferation. Several feedback loops can promote MEKi resistance, and there is considerable cross-talk between components of the Ras/ERK signalling pathway involving nearly all major signalling cascades (Neuzillet et al. 2014). ERK activation can inhibit apoptosis induced by the death receptors of the ligands FAS1, TRAIL, and TNF. In addition, aberrant activation of the Ras/ERK pathway contributes to evasion of cell senescence by upregulating the expression of telomerase. The Ras/ERK pathway is also required for epithelial–mesenchymal transition (EMT) and contributes to the maintenance of an undifferentiated or mesenchymal state

in the tumour microenvironment (TME). Specifically, the Ras/ERK pathway was shown to cooperate with the TGF-β/SMAD pathway in upregulating EMT-related genes (Maurer et al. 2011).

PI3K/AKT

PI3K/AKT(/mTOR) signalling is an important event in colorectal carcinogenesis, with mutations in *PIK3CA*, the catalytic subunit of PI3K, found in > 30% of solid malignancies (Samuels and Ericson 2006). Constitutively, *PTEN* directly inhibits PI3K/AKT signalling by dephosphorylating a key second messenger, thereby blocking cell cycle progression and inducing apoptosis. *PTEN* inactivation frequently involves promoter hyper-methylation resulting from genomic instability, explaining the correlation between MSI and *PTEN* loss (Goel et al. 2004). Functional analyses of *PIK3CA* mutations revealed that its enzymatic activity was increased, stimulating AKT/GSK-3β signalling and resulting in growth factor-independent growth, as well as invasion and metastasis (Samuels et al. 2004). *PIK3CA* mutations have been found to occur when benign colorectal tumour cells acquire the ability to invade, i.e., at the adenoma–carcinoma transition (Ijichi et al. 2001).

TGF-β/SMAD

The TGF-β/SMAD signalling pathway plays an important role in colorectal carcinogenesis, although the role of TGF-β in carcinogenesis depends on its stage (Ijichi et al. 2001). TGF-β, a prototype of a family of secreted polypeptides, regulates a wide variety of biological activities, including cell growth, differentiation, and apoptosis; extracellular matrix (ECM) production; and immune function. Members of the TGF-β superfamily form a heteromerically organized receptor complex with types I and II surface receptors, transducing intracellular signals via the SMAD complex (Pai et al. 2004).

Notch

Overexpression or constitutive activation of the Notch signalling pathway may be mechanistically involved in colorectal carcinogenesis (Qiao and Wong 2009). Alternatively, STRAP disassembles the PRC2, resulting in the activation of Notch signalling via epigenetic modification (Jin et al. 2017). A novel STRAP–Notch1–HES1 molecular axis has been shown to act as a CSC regulator in CRC, whereas DAB1 under-expression was found to suppress tumour invasion and metastasis in Notch signalling-activated mice (Jin et al. 2017; Sonoshita et al. 2015). Activation of the Notch

pathway may be associated with a loss of FBXW7, resulting in the development of adenomas in mice aged 9–10 months (Babaei-Jadidi et al. 2011).

Hedgehog

The biological activity of the hedgehog (HH) signalling pathway involves signalling that terminates at glioma-associated oncogene (GLI) transcription factors, alternating between activator and repressor forms. The important components of this pathway include the hedgehog ligands (SHH), patched (PTCH) receptor, smoothened (SMO), suppressor of fused (SuFu), and GLI transcription factors (Niyaz et al. 2019). Activation of the SHH–GLI1 pathway correlates positively with colorectal tumour development, suggesting that this pathway is activated during colorectal carcinogenesis. The expression of components of the HH pathway varies during the progression from colon adenoma to carcinoma, with the expression of SMOH and GLI1 similarly showing gradual increases from normal colon to adenoma to CRC (Xu et al. 2016). The levels of expression of SHH, PTCH, and GLI1 were found to be higher in patients with Peutz–Jeghers syndrome than in normal tissue, showing gradual increases during the adenoma-to-carcinoma sequence.

TRAIL

The TRAIL pathway, which includes death inducing signalling complex (DISC), FAS-associated protein with death domain (FADD) and pro-caspase-8 interactions, involves signalling through death receptors and the induction of apoptosis (Farooqi et al. 2019). TRAIL pathway practically indicates TRAIL-resistant one caused by deficient TRAIL death receptor transport to the cell surface. Although loss of function of TRAIL-receptor genes by mutations or methylation is not frequently found in CRC, expression of members of the IAP families, including survivin and XIAP, can contribute to TRAIL resistance in CRC (van Geelen et al. 2004). TRAIL sensitivity during colorectal carcinogenesis was previously attributed to changes in the balance between the TRAIL receptors TRAIL-R1/R2 and the decoy receptors TRAIL-R3/R4 during the progression of malignancy (Hague et al. 2005).

TP53

TP53 encoding p53 protein is a critical transcription factor that suppresses colorectal carcinogenesis through major pathways, acting as a "guardian of the genome" to maintain the integrity of DNA (Lane 1992). The p53 protein has been shown to further facilitate components of the DNA repair machinery and to directly trans-activate apoptosis-associated genes, including Bax, Puma, and Noxa, while suppressing tumourigenic and anti-apoptotic genes, such as survivin and Pdk2 (Toledo and Wahl 2006). In addition, p53 was shown to activate caspase-8 pathways through the activation of cell death receptors, such as FAS, DR5, and PIDD. *TP53* is located on the short arm of chromosome 17p13.1, and its central sequence-specific DNA-binding domain (codons 101–306) allows binding of p53 to DNA. Mutations in *TP53*, which are frequent in colorectal tumours, impede the physiological function of p53 (Toledo and Wahl 2006). The E3 ubiquitin–protein ligase MDM2 is one of the central enzymes that labels p53 with ubiquitin, maintaining p53 under-expression under normal physiological conditions (Cheok and Lane 2017). p53 protein activates p21 (WAF1), a cyclin-dependent kinase (CDK) inhibitor, which is involved in inhibiting cell transition from G1 to S phase. Mutations in *TP53* appear to occur during later stages of colon adenoma-to-carcinoma progression (Pino and Chung 2010).

The major routes of colorectal carcinogenesis

Current knowledge of the genome suggests that colorectal carcinogenesis proceeds along well-known canonical routes, constituting the adenoma-to-carcinoma sequence. The major routes of CRC evolution, namely the CIN and MSI routes, can be distinguished by their site-specificity, being predominant in the left and right colon, respectively, whereas the third route of CRC evolution, the serrated route, is located on both sides of the colon (Fig. 2).

Chromosomal instability route

More than 70% of CRCs develop through CIN routes, involving somatic mutations in *APC*, accompanied by chromosomal changes, including somatic copy number alterations (SCNAs) caused by aneuploidy, insertion–deletion mutants (in-dels), amplifications, and/or loss of heterozygosity (LOH) (Fig. 2A) (Pino and Chung 2010; Nguyen et al. 2020). However, next-generation sequencing (NGS) recently showed that the mutation profiles of CIN and microsatellite-and-chromosomal-stable (MACS) CRCs were similar, although the causes of aneuploidy or retention of diploidy could not be determined (Ham-Karim et al. 2019). The areas of genomic alterations frequently harbour mutations, leading to the activation of KRAS, the loss of p53, and LOH of genes on the long arm of chromosome 18 (Markowitz and Bertagnolli 2009). Increased structural defects associated with CIN presumably expand the repertoire of driver

Fig. 2 The three major routes of colorectal carcinogenesis. **A** The chromosomal instability route (CIN). **B** The microsatellite instability route (MSI). **C** The serrated route, involving either traditional serrated adenomas (TSA) or sessile serrated adenomas (SSAs). [a]MSI polyps included all polyps, whether adenomatous, sessile, or hamartomatous
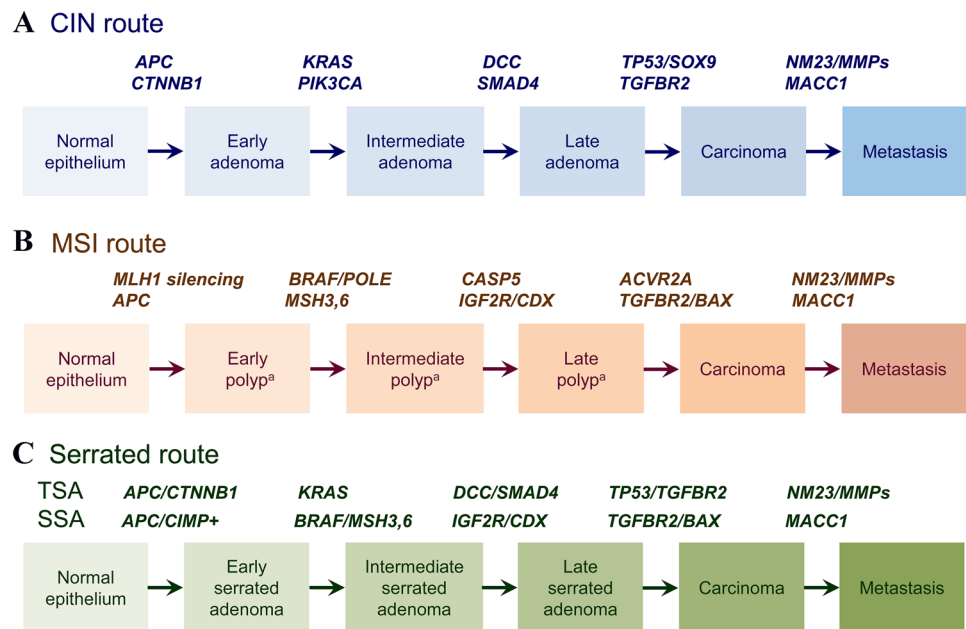
mutations promoting carcinogenesis. Few *MYC* mutations have been observed in CRCs, but *MYC* amplifications have been reported in approximately 10% of CRCs, resulting in a feature associated with poorer outcomes (Kozma et al. 1994). CRCs that develop through the CIN route are positively associated with the risk of developing metastases, whereas those that develop through the MSI route are not (Angius et al. 2021). In some patients with polyposis, the hamartoma–carcinoma sequence could be explained by three potential steps associated with cellular and molecular pathogenesis, namely dysplastic transformation, altered turnover rate of stem cell lineage, and hamartoma–adenoma transition (Bosman 1999; Korsse et al. 2013; Jansen et al. 2009). Hamartoma–dysplasia transformation was associated with stepwise alterations of *SMAD4/STK11*, along with loss of TGF/β-catenin signalling, whereas hamartoma–adenoma transition included mixed hamartomatous and adenomatous components in a polyp, or mixed clusters of hamartoma and adenoma in an individual.

Deficient mismatch repair route

Microsatellite instability (MSI) is a feature observed in approximately 10–20% of CRCs (Fig. 2B). MSI in colorectal tumours primarily arises from dysfunction of the mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*, and leads to numerous mutations, in particular within highly repetitive microsatellite regions (Farooqi et al. 2019). Alternatively, deletions in the 3′ end of *EPCAM*, a gene located 17 Kb upstream of *MSH2*, eliminate termination signals, resulting in *MSH2* promoter hyper-methylation with epigenetic silencing

(Tutlewska et al. 2013). Although *EPCAM* mutations are rare, enhanced EPCAM expression, which correlates with the downregulation of E-cadherin, has oncogenic potential linked to CSCs and EMT, resulting in poor differentiation, vascular and marginal invasion, and lymph node metastasis (Kempers et al. 2011). Germline mutations in MMR genes are rare in CRCs, except in patients with Lynch syndrome (LS). MSI-containing CRCs have been associated with the *BRAF* V600E mutation, which has been observed in around 80–90% of sporadic MSI-H CRCs but rarely in CRCs due to LS (Müller et al. 2016). Another deficient MMR caused by *MLH1* promoter hypermethylation may also be useful in distinguishing sporadic from LS-associated CRCs. Up to 90% of MSI CRCs carry *TGFBR2* mutations, which cannot prevent cellular proliferation (Pinheiro et al. 2015). Other genes disrupted by MSI included genes encoding proteins that regulate proliferation (e.g., *GRB1*, *TCF4*, *WISP3*, *ACVR2*, *IGF2R*, *AXIN2*, and *CDX*), cell cycle arrest or apoptosis (e.g., *CASP5*, *PRDM2*, *BCL10*, *PTEN*, *PA2G4*, and *FAS*), and DNA repair (e.g., *MBD4*, *BLM*, *CHK1*, *RAD50*, *MSH3*, and *MSH6*), although most of these gene alterations were not associated with major functional consequences (Guinney et al. 2015). *APC* mutations have been found in 35–50% of MSI tumours, suggesting that the initiating event in adenoma formation may be shared by MSI and CIN tumours without showing any correlations, distinguishing these tumours from MSI tumours initiated by *BRAF* mutations (Müller et al. 2016). Other CRCs may be distinguished by genome stability lacking hypermutation and aneuploidy, but enriched in DNA hypermethylation and mutations in *KRAS*, *SOX9*, and *PCBP1* (Liu et al. 2018).

Serrated route

Approximately 10–20% of CRCs may develop through a different sequence of morphological changes, known as the serrated route (Fig. 2C). Although the majority of serrated polyps are hyperplastic, without malignant transformation, a subset of serrated lesions can progress to CRCs (Nguyen et al. 2020). Based on genetic and morphological pathogenesis, the serrated route involves dual traits from either the CIN or deficient MMR pathways, producing two types of premalignant precursor lesions, traditional serrated adenomas (TSAs) and sessile serrated adenomas (SSAs), respectively (Müller et al. 2016). Approximately 80% of TSAs carry *KRAS* mutations, whereas approximately 80% of SSAs carry *BRAF* and MMR-associated mutations or gene silencing caused by promoter hypermethylation (Ham-Karim et al. 2019). The latter phenotype frequently includes *MGMT* promoter methylation as a consequence of the low-level MSI (MSI-L) phenotype. The characteristic morphological features of TSAs include architectural dysplasia with ectopic crypt formation and serration, likely associated with molecular alterations that result in hyper-proliferation and inhibition of apoptosis (Nguyen et al. 2020). TSAs, which are diagnosed based on their characteristic cytology (eosinophilic cytoplasm and central, elongated hyperchromatic nuclei) and slit-like epithelial serrations with ectopic crypt formation, may progress to adenocarcinoma (Davies et al. 2002). By contrast, SSAs have *BRAF* mutations and MSI, which correlate with mucinous or poorly differentiated tumours, and may progress to serrated or mucinous adenocarcinomas (Advani et al. 2018). These tumours are frequently found in the right colon, and predominantly in older and female patients.

Hereditary colorectal cancer

Approximately 5% and 20% of all CRCs have been found to be familial and hereditary CRCs (FCRC and HCRC), respectively. The former category includes a variety of genetically verified syndromes with high penetrance, whereas the latter can be applied to any familial occurrences of CRC due to multi-genic variants, each with low-level effects as in the analysis of polygenic risk scores (Crouch and Bodmer 2020). Two dominant routes of colorectal carcinogenesis were revealed through analysis of patients with LS and FAP. LS has been erroneously called hereditary nonpolyposis CRC, but "nonpolyposis" is a misnomer as almost all colorectal polyps can be LS precursor lesions, which typically present with villous growth and high-grade dysplasia (Burt et al. 2004). Recently, the classical perspectives of LS as an "accelerating" disease have been challenged to provide many alternatives to classical adenoma–carcinoma sequence,

importantly by the discovery of MMR-deficient crypt foci (MMR-DCF) (Ahadova et al. 2018). Ahadova et al. suggested LS CRCs to be possibly developed through three distinct pathways, i.e., MMR-proficient adenomas after secondary inactivation of the MMR system (pathway 1), the other larger parts from precursor lesions in which MMR deficiency is an early event, likely to include MMR-DCF, either through an adenomatous phase (pathway 2) or as non-polypus lesions with immediate invasive growth (pathway 3). Although there are some genotypic and phenotypic overlaps, FCRC represents 14 syndromes, including six LS and LS-associated spectra and eight inherited polyposis syndromes. The former category includes traditional LS, Muir–Torre syndrome, Turcot syndrome, constitutional MMR deficiency (CMMRD) syndrome, EPCAM-associated LS (EALS), and familial CRC type X (FCCTX). The latter category includes a broad spectrum of inherited polyposis syndromes, including FAP, *MUTYH*-associated polyposis (MAP), polymerase proofreading-associated polyposis (PPAP), serrated polyposis syndrome (SPS), hereditary hamartomatous polyposis including Peutz–Jeghers syndrome (PJS), juvenile polyposis syndrome (JPS), PTEN hamartoma tumour syndrome, and hereditary mixed polyposis syndrome. The lifetime risks of associated neoplasms in patients with FCRC are summarized in Table 1.

Lynch syndrome and associated spectra

Men and women with LS have estimated lifetime risks for CRC of 70% and 40%, respectively (Wells and Wise 2017). Endometrial adenocarcinoma is the most common extra-colonic cancer, with a lifetime risk of 32–45%, followed by ovarian, small bowel, gastric, urinary tract, pancreas, and brain cancers, in that order. Variants in *MLH1*, *MSH2*, *MSH6*, and *PMS2* were found in 40%, 34%, 18%, and 8%, respectively, of patients with LS (Peltomäki et al. 2020). *EPCAM* deletions are also considered a cause of LS, being present in up to 30% of patients with *MSH2*-mutation (−) tumours and 20% of those without MMR mutations (Tutlewska et al. 2013). *EPCAM*-associated LS carries a risk of CRC, with the phenotype of these tumours being similar to those of tumours with *MLH1* and *MSH2* mutations, whereas the cumulative risk of endometrial cancer in patients with *EPCAM*-associated LS is much lower (Tutlewska et al. 2013; Kempers et al. 2011). Muir–Torre syndrome and Turcot syndrome are particular LS subtypes caused by MMR alterations, selectively displaying sebaceous neoplasms of the skin and brain tumours, respectively. Muir–Torre syndrome carries *MLH1*, *MSH2*, and *MSH6* mutations, along with several recently described autosomal recessive traits, including *MUTYH* alterations in the absence of MSI (Gadish et al. 2005). LS and FAP can co-segregate with Turcot syndrome, mostly inherited through autosomal recessive transmission

Table 1 Causative genes and associated neoplasms of familial colorectal cancer

Category/syndrome	Causative genes	Cytogenetic banding	Lifetime syndrome risks of associated cancers[a] (%)									
			Colon	Stomach	SB	Pancreas	Breast	EM	Ovary	Urinary	Thyroid	Others
Lynch syndrome or LS-associated spectra												
LS[b]	MMR[c]	MMR[c]	40–70	6–13	1–4	1–4		32–45	4–12	5–21		
		MLH1/MSH2[d]	50	6–13	1–4	1–4		25/49	4–12	5–21		
		MSH6[d]	24	6–13	1–4	1–4		61	4–12	5–21		
		PMS2[d]	14					13				
Muir–Torre[b]	MMR[c]/MUTYH	MMR[b]/1p34.1	40–70	6–13	1–4	1–4		15	4–12	5–21		Skin
Turcot[b]	MMR[c]/APC	MMR[b]/5q22.2	40–70	6–13	1–4/	1–4		32–45	4–12	5–21	5–20	GB/MB
CMMRD	PMS2/MSH6	MMR[c]	32 (colon + stomach + SB)	6–13								GB, H
EALS	EPCAM	2p21	40–70	6–13	Moderately increased risk of CRC/lower risk of extra-colonic cancers							
FCCTX	Unidentified	Unidentified	Moderately increased risk of CRC/lower risk of extra-colonic cancers									
Inherited polyposis syndrome												
FAP	APC	5q22.2	90 (69[e])	2–5	5–20	2–5					5–20	MB
MAP	MUTYH	1p34.1	43–63	Less common than those in FAP, otherwise a similar spectrum to LS								
PPAP	POLE/POLD1	12q24.33/19q13.3	70		9.5			12	IR			
SPS	RNF43	17q22	20	Undetermined								
PJS	STK11	19p13.3	40	5–20	2–5	5–20	50	10	20			
JPS	SMAD4/BMPR1A	18q21.2/10q23.2	20–40	5–20								
PHTS	PTEN	10q23.31	9				85	28		34	35	Melanoma
HMPS	GREM1	15q13.3	Undetermined									

SB small bowel, EM endometrial, FAP familial adenomatous polyposis, MMR mismatch repair genes, LS Lynch syndrome, CMMRD constitutional MMR deficiency, EALS EPCAM-associated LS, FCCTX familial colorectal cancer type X, MAP MUTYH-associated adenomatous polyposis, SPS sessile polyposis syndrome, PJS Peutz–Jeghers syndrome, JPS juvenile polyposis syndrome, PHTS PTEN hamartoma tumour syndrome, HMPS hereditary mixed polyposis syndrome, GB/MB glioblastoma/medulloblastoma, H haematological malignancy, IR increased rate

[a] Lifetime syndrome risks mostly based on the ACS Guideline of Hereditary Gastrointestinal Cancer Syndromes (2015; https://gi.org/guidelines/) and included references; PPAP based on the National Study of Colorectal Cancer Genetics (2013); UK; PHTS based on the International Cowden Consortium (2012)

[b] Lifetime syndrome risks were generally calculated on the basis of LS with additional FAP in Turcot syndrome

[c] MMR (cytogenetic band) includes MLH1 (3p22.2), MSH2 (2p21-p16.3), MSH6 (2p16.3), and PMS2 (7p22.1)

[d] https://doi.org/10.1200/78.4777.JCO.2018.78.477; http://www.hccpjournal.com/content/7/1/17

[e] Risk in parentheses indicates lifetime risk (%) in attenuated FAP

with bi-allelic *MLH1*/*PMS*2 and *APC* mutations, respectively (Gadish et al. 2005). Glioblastomas occur in patients with MMR gene mutations, specifically those with *MLH1* mutations, whereas medulloblastomas have been associated with *APC* mutations. Another LS subset, a specific phenotype designated constitutional mismatch repair deficiency (CMMRD), is a highly penetrating cancer-predisposition syndrome caused by bi-allelic MMR alterations, more frequently in *PMS2* and *MSH6* than in other MMR genes (Nejadtaghi et al. 2017). Tumours frequently observed in patients with CMMRD include brain (48%), gastrointestinal (32%), and haematological (15%) tumours. As many as 40% of CRCs fulfilling the clinical criteria for LS exhibit MSS, transiently called familial colorectal cancer type X (FCCTX) (Nejadtaghi et al. 2017). Causative genes for FCCTX remain to be verified, although several candidate genes have been proposed by multi-gene panels (MGPs), including *AXIN2*, *BCR*, *BLM*, *BMPR1A*, *BRCA1*, *BRCA2*, *BRF1*, *CHEK2*, *FAN1*, *GABBR2*, *GALNT12*, *HABP4*, *KIF24*, *MSH3*, *MUTYH*, *OGG1*, *POLD1*, *RPS20*, *SEMA4A*, and *ZNF367* (Hansen et al. 2017; Yurgelun et al. 2015a, b; Gupta et al. 2019).

Inherited polyposis syndrome

Inherited polyposis syndrome can be stratified by histological morphology as adenomatous, hamartomatous, and mixed polyposis. Germline *APC* mutations around codon 1300 (codons 1286–1513) have been associated with severe colorectal polyposis of FAP (Fearnhead et al. 2001). Otherwise, somatic *APC* mutations of codons 1400–1580 have been observed in upper gastrointestinal polyps, including severe duodenal polyposis clusters, with these *APC* gene products retaining only one of the 20-amino acid β-catenin-binding degradation repeats (Groves et al. 2002). MAP tends to present later in life (age > 25 years) than FAP, and develops predominantly in the proximal colon. Histologically, these lesions are mucin-rich, with abundant lymphocyte infiltration, and patients with MAP have a better prognosis than patients with sporadic CRCs (Kanth et al. 2017). The two most common *MUTYH* founder mutations, Y179C and G396D (previously called Y165C and G382D, respectively), are present in 70–80% of individuals of Northwestern European ancestry with MAP, with these mutations inherited in an autosomal recessive manner (Stoffel and Boland 2015). However, approximately one-third of individuals with biallelic *MUTYH* mutations develop CRC in the absence of polyposis, suggesting incomplete penetrance (Sereno et al. 2014).

Germline pathogenic variants in the exonuclease domain (ED) of polymerases POLE1 and POLD predispose to PPAP (Church et al. 2013). Most *POLE* variant heterozygotes carry a colorectal tumour phenotype, with their median ages at diagnosis of polyps and CRCs being 36 and 44 years, respectively (Palles et al. 2021). Endometrial and ovarian cancers are the most common malignancies found in women with *POLD1* variant heterozygotes under age 50 years (Church et al. 2013; Palles et al. 2021). The updated diagnostic criteria for SPS (WHO, 2019) include ≥ 5 serrated polyps above the rectum that are ≥ 5 mm in diameter with at least two ≥ 10 mm polyps, or ≥ 20 serrated polyps of any size throughout the colon with ≥ 5 above the rectum, along with increased risk of CRC (Stanich and Pearlman 2019). Nonsynonymous mutations in *RNF43*, encoding E3 ubiquitin-protein ligase, have been observed only in the affected siblings of patients with SPS, suggesting that these mutations are causative genetic variants (Quintana et al. 2018). *NTHL1* tumour syndrome, a novel type of familial CRC predisposing to adenomatous polyposis and CRC, is caused by germline bi-allelic pathogenic variants in *NTHL1* (Weren et al. 2015). This condition is also accompanied by increased lifetime risks for breast cancer and variable extra-colonic tumours of both the LS- and FAP-associated spectra.

The Peutz–Jeghers syndrome (PJS) phenotype manifests when germline *STK11* (*LKB1*) mutations, inherited in an autosomal-dominant manner, are accompanied by acquired defects in the other allele in somatic cells (Tacheci et al. 2021). JPS is caused by *SMAD4* and *BMPR1A* (*ALK3*) mutations, inherited in an autosomal-dominant manner with incomplete penetrance, with collective rates of 23 and 21–38%, respectively (Woodford-Richens et al. 2000a, b; Zhou et al. 2001). *BMPR1A* is located upstream of *SMAD4* in the TGF-β pathway, and mutations encoding BMP receptors result in loss of intracellular BMP signalling via SMAD 4 (Chow and Macrae 2005). In contrast to the initial landscaper-defect hypothesis of JPS (Kinzler and Vogelstein 1998), fluorescence in situ hybridization showed that epithelial malignancies in JPS are likely to develop through direct progression in the epithelial components of hamartomas, suggesting that SMAD4 acts as a gatekeeper in both JPS and sporadic cancers (Woodford-Richens et al. 2000). Patients with *PTEN* hamartoma tumour syndrome (PHTS), including those with Bannayan–Riley–Ruvalcaba syndrome, Cowden syndrome, Gorlin syndrome, and Proteus-like syndrome, are at increased risk of developing cancer (Hendricks et al. 2021). Mutations in *PTEN* (10q23.3) and *PTCH* (9q31) can rule out almost all JPS patients that are exclusively considered in the context of PHTS (Zhou et al. 2001). Patients with Cowden syndrome and similar phenotypes may also have associated hypermethylation of the *KLLN* promoter, deregulating p53-induced apoptosis (Nizialek et al. 2015).

Hereditary mixed polyposis syndrome (HMPS) is characterized by a small number of polyps with mixed phenotypes, most commonly adenoma and non-dysplastic mixed serrated/inflammatory polyps. The causative genetic alteration in HMPS was found to be a 40 Kb duplication at the 3' end

of the *SCG5* (Ashkenazi Jewish founder mutation), resulting in aberrant epithelial expression of the mesenchymal BMP antagonist, Gremlin, transmitted via autosomal-dominant inheritance (Davis et al. 2015).

Genotype–phenotype correlations and causative gene discovery

Particular traits of CRC carcinogenesis have been investigated by evaluating genotype–phenotype correlations. Approximately 16% of CRC patients aged < 50 years (early-onset CRC, EOC) were found to carry at least one pathogenic cancer-susceptible gene mutation (Pearlman et al. 2017). One NGS study according to the revised Bethesda guidelines found that *FAT4* mutation rates were lower in patients with EOC than in those with later-onset CRCs, potentially defining an early-onset MSS subtype (Kim et al. 2021). Another EOC study by the polygenic risk score demonstrated that the cumulative burden of common genetic variants associated with CRCs was higher in patients with EOCs, particularly in the absence of a family history (Archambault et al. 2020). Furthermore, EOCs tend to present with higher histological grade and higher rates of recurrence and metastasis. The incidence of CRC is modestly higher in patients with Li–Fraumeni syndrome, characterized by germline mutations in *TP53*, than in the overall population. One registry-based analysis found that germline alterations in *TP53* were associated with EOCs (Yurgelun et al. 2015a, b). Taken together, these findings indicated that EOCs are not only associated with rare monogenic or high-penetrance genetic syndromes in high-risk families, but also with low-penetrant multi-gene variants. The morphology and histology of left-sided (LCRC) and right-sided (RCRC) CRCs also differ markedly. Epidemiologically, younger individuals and men are more likely to have LCRC, whereas elderly persons and women are more likely to develop RCRC. Nevertheless, LCRC remains the most commonly diagnosed form of CRC across all age groups, constituting approximately 70% of all CRCs (Jess et al. 2013).

Most comprehensive MGPs have utilized NGS to identify pathogenic single or multiple gene variants. However, gene discovery via MGPs identifies considerable numbers of variants of unknown significance (VUS), as well as clinically questionable or non-actionable variants (Hall et al. 2014). Actionable CRC gene variants identified by MGPs include mutations/alterations in *APC* (I1307K polymorphism), *AXIN2*, *CHEK2*, *GREM1*, *GALANT12*, *MSH3*, *MUTYH* (monoallelic), *NTHL1*, *POLD1*, and *POLE* (Gupta et al. 2019) A genome-wide association study (GWAS) of a Finnish cohort of patients with CRC found an association between the intronic SNP rs992157 at 2q35 with *PNKD/TMBIM1*, a finding independently replicated in a

meta-analysis of CRC patients of European ancestry (Tanskanen et al. 2018). Another study demonstrated that *TRIM4* and *PYGL*, which encode proteins that influence redox homeostasis and cellular metabolic reprogramming, respectively, may be implicated in a novel CRC pathway linked to cell growth and proliferation (Bien et al. 2019). Another custom-made MGP (HaloPlex®) targeting 112 genes, covering previously identified and candidate CRC susceptibility genes, identified 17 pathogenic variants as potential gene alterations associated with CRC susceptibility; these included variants of *MUTYH*, *ATM*, *AXIN1*, *AXIN2*, *BRCA1*, *CHEK2*, *BMP4*, *CCDC18*, *NUDT7*, *PICALM*, *PTPRJ*, *SLC5A9*, *TLR2*, *TWSG1*, *UBAP2*, *USP6NL*, and *ZFP14* (Hansen et al. 2017). New approaches designed to identify rare variants should herein consider the two main criteria suggested by the historical overview, i.e., genes in which obviously severe disruption of function gives rise to a severe, usually familial, version of the disease being studied and genes known to be involved in the biology of the disease based on biochemical and physiological analyses (Bodmer and Bonilla 2008).

Consensus molecular subtype

Classification of CRCs by consensus molecular subtype (CMS) may assist in the development of personalized medicine to treat these patients. These classifications, however, have limitations, mainly confined to MSI, mesenchymal cells, and specific driver gene mutations. Additionally, regional tumour heterogeneity in molecular classifiers, particularly concerning CMS4 with a stromal mixture, can result in tumour misclassification (Dunne et al. 2016). CMS nevertheless appears to increase understanding of the molecular and immune signatures that predict clinical behaviour and responses to different therapeutic agents (Farooqi et al. 2019). CMS1 tumours are characterized by widespread hypermethylation, whereas CMS2-4 tumours show development via the CIN route, as measured by SCNA counts (Guinney et al. 2015). CMS1 CRCs are hyper-mutated tumours with MSI due to defective MMR, usually caused by *MLH1* promoter hypermethylation. Nearly 80–90% of sporadic hyper-mutated CRCs also carry *BRAF* V600E mutations. *POLE* or *POLD1* ED (proofreading) mutations insert incorrect nucleotides during DNA replication, resulting in an ultra-mutated phenotype. Additionally, gene expression profiling has shown evidence of strong immune activation in CMS1, with high tumour infiltration by CD8+ cytotoxic T lymphocytes. CMS2 CRCs display epithelial differentiation and strong upregulation of Wnt and MYC downstream targets, classically implicated in colorectal carcinogenesis. By contrast, multiple metabolic signatures are enriched in CMS3 CRCs, in agreement with the occurrence of *KRAS*

activating mutations that induce metabolic adaptations. Finally, CMS4 CRCs show clear upregulation of genes implicated in EMT and signatures associated with the activation of the TGF-β signalling, angiogenesis, and matrix remodelling pathways, and activation of serum complement. Finally, mixed CMS subtypes, probably resulting from multiple clones in an individual tumour, have been detected in as many as 13% of CRCs, although these are considered to be outliers. Irrespective of patient cohort, patients with CMS4 tumours have poorer survival outcomes, whereas the percentage of long-term survivors is higher in patients with CMS2 tumours than in those with other subtypes. Interestingly, survival in patients with CMS1 tumours is very poor after relapse, in agreement with studies showing a poorer prognosis in patients with MSI and *BRAF*-mutated CRCs following tumour recurrence (Guinney et al. 2015).

A study correlating CMS subtypes with tumour site suggested that the mutational profiles of tumours in the transverse colon differed from the profiles of right-sided, and especially left-sided, tumours (Loree et al. 2018). Concerning EOC, possibly related to heredity, CMS1 was the most common subtype, whereas CMS3 and CMS4 were uncommon, whereas CMS2 was relatively stable across age groups (Willauer et al. 2019). The CMS classification holds clear potential for clinical use in predicting both prognosis and response to systemic therapy, which seems to be independent of the classifier used (Ten Hoorn et al. 2021). Although fluoropyrimidine-monotherapy lacked benefits for dMMR CMS1 tumours, whereas additional bevacizumab seemed beneficial and immune checkpoint inhibitors received a new agnostic indication for unresectable or metastatic CRCs with high tumour mutational burden in CMS1. Adjuvant chemotherapy in stage II and III CRC increased overall survival in CMS2 and CMS3, although not effective in CMS4 tumors. However, CMS4 metastatic CRC predicted benefits from irinotecan combined with cetuximab (in *KRAS* wt tumours) and bevacizumab (in *KRAS* mutant tumours), recommended as preferred first-line options in CMS4 metastatic CRC patients. On the other hand, CRC microbiomes might be associated CMS, for example, elevated abundance of *Fusobacterium*, *Peptostreptococcus*, or *Parvimonas*, to be associated with CMS1, underscored the potential role of oral poly-microbial communities in the development of a subset of CRC pathogenesis (Purcell et al. 2017).

A recent global transcriptomic immune classification proposed six immune subtypes (ISs), along with their interplay with CMS types (Soldevilla et al. 2019). Only five ISs, all but C5, were identified in CRCs, with C1 (wound healing subtype, 77%) and C2 (IFN-γ dominant subtype, 17%) being the most frequent. CMS1 showed the highest proportion of C2 (53%), whereas C1 was particularly frequent in CMS2 (91%). CMS3 had the highest representation of C3 (inflammatory subtype, 7%) and C4 (lymphocyte depleted subtype, 4%), whereas all

C6 TGF-β dominant subsets belonged to CMS4 (2.3%). The immunologically quiescent C5 subtype showed the lowest lymphocyte and highest macrophage responses, dominated by M2 macrophages. Another study found that 54 (87%) of 62 colorectal adenomas could be classified according to the CMS (Komor et al. 2018). The metabolic type of CMS3, which was least common among CRCs, was the most prevalent among adenomas (73%), followed by CMS2 (13%) and CMS1 (2%). None of the adenomas presented with the mesenchymal subtype CMS4, consistent with the lack of invasion-associated stroma in adenomas.

Concluding remarks

Current knowledge of genomic colorectal carcinogenesis is generally based on the adenoma-to-carcinoma sequence, and to some extent on the hamartoma–carcinoma sequence, serrated neoplasia, and LS pathway. The classical routes of carcinogenesis, via CIN, MSI, and serrated transition have been identified, each exhibiting site-specificity and distinctive tumour biology. Although the events initiating adenoma formation may be common to MSI and CIN tumours, TSAs and SSAs are confined to the serrated route, with most carrying *KRAS* mutations and MSI, respectively. Post-transcriptional modifications also participate in oncogenic transformation throughout all the steps of carcinogenesis, including DNA methylation and dysregulation of histone modifications. Environmental factors, including alterations of the enteric microbiome, participate in the genomic orchestration of colorectal carcinogenesis through gene–environment interactions. FCRCs have played important roles in determining aspects of colorectal carcinogenesis. Recent advances in the NGS platform may define the genes involved in FCRC by, for example, determining FCCTX using comprehensive MGPs.

Declarations

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