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Solid Tumour Section

Review

Colon: Colorectal adenocarcinoma

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Classification

Colorectal Cancer (CRC) can be classified from the molecular or etiological point of view:

Molecular pathway

- Chromosome instability (CIN)
- 85% of all CRCs
- Somatic inactivation of APC, p53;
- Microsatellite instability (MSI)
- 15% of all CRCs
- Hypermethylation or somatic inactivation of DNA repair enzymes;

Etiology

- Sporadic CRC
- 84% microsatellite stable (MSS) CRC
- 10% microsatellite instable (MSI) CRC;
- Hereditary CRC
- 1% Familial Adenomatous Polyposis (FAP) (MSS CRC)
- 5% Hereditary Non-Polyposis Colorectal Cancer (HNPCC) (MSI CRC).

In the last years, downstream molecules of different pathways such as KRAS and BRAF are included in proposals for a more differentiated molecular classification of CRC.

The CpG Methylator Phenotype (CIMP) status also seems to play an important role in the pathogenesis of CRC.

Clinics and pathology

Etiology

According to the WHO Classification of Tumors of the Digestive System the following factors may play an etiological role in the development of CRC:

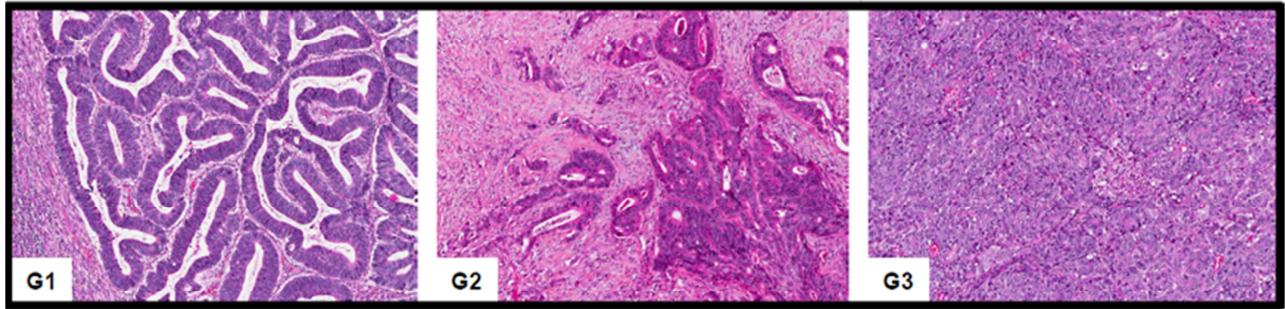
- Diet (high caloric food, meat consumption)
- Obesity
- Sedentary lifestyle
- Smoking
- Alcohol consumption
- Inflammatory Bowel Disease (IBD)
- Cohn's disease
- Ulcerative colitis.

Epidemiology

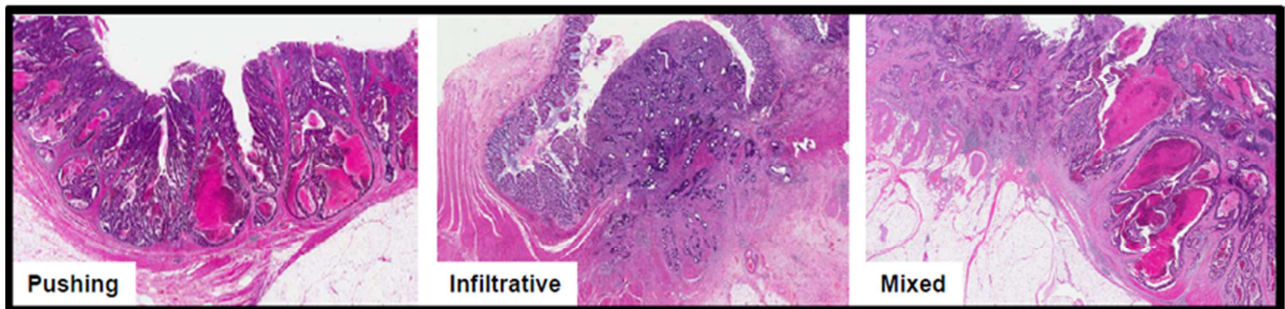
The following epidemiologic data are based on the WHO Classification of Tumors of the Digestive System:

- In 2008, 1.23 million new CRC cases occurred worldwide (9.7% of all new cancers).
- CRC is the 4th most frequent cancer in men and 3rd in women.
- Higher rates occur in industrialized, high-resource countries (40-60 per 100000).
- Incidence increases with age.
- Rates of colon cancer are about 20% higher and rectal cancer about 50% higher in men than in women.
- Worldwide mortality rate is about 50% of the incidence rate.

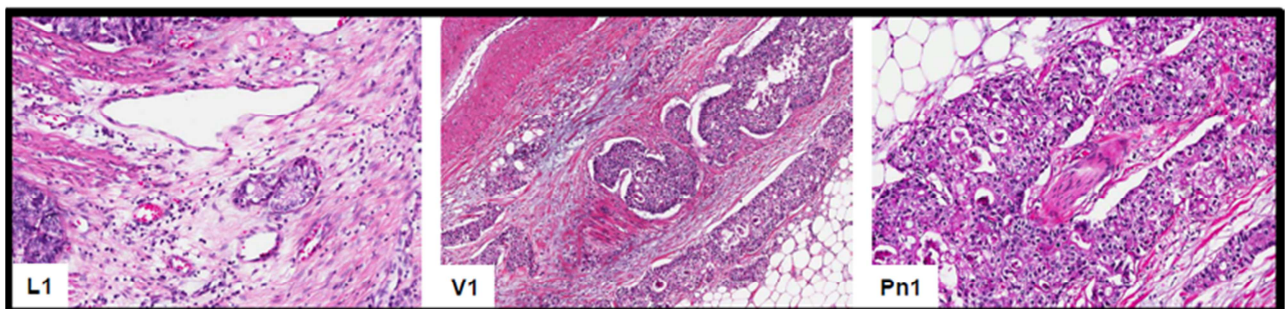
Tumor grade (100x)



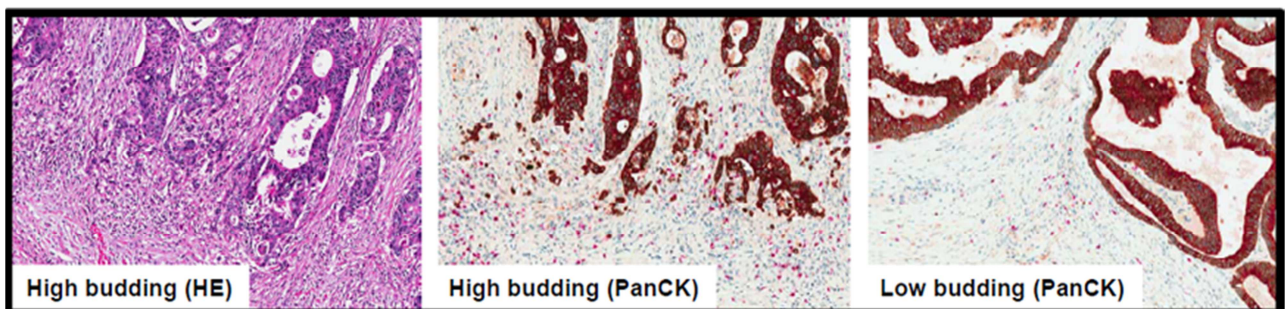
Tumor border configuration (10x)



Prognostic factors: Lymphovascular and perineural invasion (150x)



Additional prognostic factors: Tumor budding (150x)



Clinics

- Change bowel habits including constipation and diarrhea
- Melena
- Abdominal pain
- Anemia due to chronic low-grade bleeding
- Weight loss
- Fever

- Malaise.

Pathology

The histological diagnosis of CRC is based on the invasion through the muscularis mucosae into the submucosa.

The different histological subtypes include:

- Adenocarcinoma
- Mucinous adenocarcinoma

- Signet ring cell carcinoma
- Medullary carcinoma
- Serrated adenocarcinoma
- Cribriform comedo-type adenocarcinoma
- Micropapillary adenocarcinoma
- Adenosquamous carcinoma
- Spindle cell carcinoma
- Squamous cell carcinoma
- Undifferentiated carcinoma.

The gold standard for staging is the TNM classification proposed by the UICC/AJCC.

Important parameters included in the TNM classification are:

- T : Primary Tumor
- N: Regional Lymph Nodes
- M: Distant Metastasis
- G: Histopathological grading
- L: Lymphatic invasion
- V: Venous invasion
- Pn: Perineural invasion.

Molecular features in Pathology that can be important in the daily diagnostic practice are:

- Microsatellite status: Determined by immunohistochemistry and/or PCR
- KRAS status: Determined by PCR
- BRAF status: Determined by PCR.

Treatment

Surgery is the primary therapy of CRC.

Adjuvant therapy:

- Colon Cancer: Chemotherapy
- Rectal Cancer: Chemo- and radiotherapy
- Anti-EGFR therapy: dependent on the KRAS mutational status.

Prognosis

The prognosis is mainly based on the TNM staging system (see Pathology section).

Additional prognostic factors can be:

- Tumor border configuration
- Tumor budding
- Tumor regression grade
- Bowel perforation
- Intra- and peritumoral inflammation
- Circumferential resection margin
- Microsatellite status
- BRAF
- EGFR
- VEGF
- Loss of heterozygosity
- CEA level
- miRNA
- CIMP status.

Cytogenetics

Cytogenetics Morphological

There are two types of colorectal cancers, according to the ploidy:

Aneuploid tumors showing numerous allelic losses; Aneuploidy, loss and rearrangements of chromosome 1p (about 70%), 5q (55%, loss of APC), 15q, 18q (65%, loss of DCC), 17p (80%, TP53), and 17q (30%); and abnormalities in 7q (25%) and 8p (55%). Reciprocal translocation t(5;10)(q22;q25), inv(5)(q22q31.3).

Diploid tumours without frequent allelic losses.

Genes involved and proteins

Note

Extensive data on the genetic background of tumor progression in colorectal cancer (CRC) is available based on molecular pathology methods including genome wide sequencing. Alterations in oncogenes and tumor suppressor genes are intricately involved in the development and progression of CRC, some of which are strong predictors of treatment response to targeted therapies and chemotherapeutics. In the following, a comprehensive overview of both hereditary and somatic genetic changes in CRC is provided including genetic changes causing DNA mismatch repair deficiency as well as genomic alterations leading to deregulation of the WNT, RTK/Ras/MAPK, PI3K, TGF-Beta and P53 signaling pathways.

Mismatch repair

MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli))

Location

3p22.3

DNA / RNA

19 exons, 2524 bp mRNA.

Protein

Involved in mismatch repair (MMR) process after DNA replication. Forms heterodimer with PMS2 (also known as MutL α). This heterodimer is responsible for downstream MMR events such as strand discrimination, recruiting proteins needed for excision and resynthesis.

Germinal mutations

Most frequent cause of Lynch syndrome (also known as HNPCC). Over 300 mutations have been described (nucleotide substitutions or insertions/substitutions) resulting in a truncated protein. Hereditary germline epimutations have also recently been reported.

The full penetrance of Lynch syndrome requires the biallelic "double hit" inactivation of the responsible MMR gene (see also MSH2, MSH6 and PMS2) for tumor development.

Somatic mutations

Mutation is rare; usually epigenetic modification due to methylation which results in MSI-H status. Methylation of MLH1 is by far the most frequent cause of sporadic MSI-H in CRC.

MLH3 (*mutL* homolog 3 (*E. coli*))**Location**

14q24.3

DNA / RNA

12 exons, 2 major mRNA transcripts: 7911 and 7839 bp.

ProteinInvolved in mismatch repair process after DNA replication. Competes against PMS2 to form heterodimer with MLH1 (*MutL γ*) which assists *MutL α* .**Germinal mutations**

Only few MLH3 germline mutations have been described in Lynch syndrome, by far most of which amino acid substitutions. Implications are still unclear. To date, there is no evidence that these germline mutations cause the full Lynch syndrome phenotype.

Somatic mutations

Shows insertions/deletions usually in A(8) repeats in MSH-H tumors. Considered to be a secondary mutation which is believed to further increase genetic instability.

MSH2 (*mutS* homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*))**Location**

2p21

DNA / RNA

16 exons, mRNA transcript 3145 bp.

ProteinInvolved in mismatch repair process after DNA replication. Binds to MSH6 or MSH3 to form the *MutS α* or *MutS β* complex. The *MutS* complex associates with the *MutL* complex composed of MLH and PMS2.**Germinal mutations**

Frequent cause of Lynch syndrome. Over 300 mutations have been described (nucleotide substitutions or insertions/substitutions) resulted in a truncated protein. Hereditary germline epimutations have also recently been reported.

Somatic mutations

Rare; sporadic MSH2 mutations have been described in sporadic MSI-H cases. Somatic methylation usually occurs as a "second hit" of the wild-type allele in cases with germline mutation.

MSH3 (*mutS* homolog 3 (*E. coli*))**Location**

5q14.1

DNA / RNA

24 exons with 2 mRNA transcripts of 5 and 3.8 kb.

ProteinInvolved in post-replicative DNA mismatch repair. Binds with MSH2 to form the *MutS β* heterodimer, which recognizes large insertion-deletion loops. Formsa ternary complex with *MutL α* after mismatch binding.**Germinal mutations**

Mostly LOH and missense variants, seen in MSI-L tumors.

Somatic mutations

Hypermethylation has been described in other tumors such as endometrial carcinoma. Functional impairment of MSH3 is probably not required for the formation of MMR-deficient tumors but is selected for during tumor progression.

MSH6 (*mutS* homolog 6 (*E. coli*))**Location**

2p16

DNA / RNA

10 exons, mRNA transcript 4435 bp.

ProteinInvolved in post-replicative DNA mismatch repair. Forms heterodimer with MSH2 (*MutS α*).**Germinal mutations**

A less frequent cause of Lynch syndrome with variable penetration. Deletion and frameshift mutation result in a truncated protein. Can be associated with so-called early-onset CRC or Lynch syndrome with atypical features, such as endometrial cancer.

Somatic mutations

Mutation or epigenetic activation is rare in CRC.

PMS2 (*PMS2* postmeiotic segregation increased 2 (*S. cerevisiae*))**Location**

7p22.1

DNA / RNA

15 exons, mRNA transcript 2851 bp.

ProteinInvolved in post-replicative DNA mismatch repair. Forms the *MutL α* complex together with MLH1.**Germinal mutations**

Mostly deletions, some missense variants, most mutations are unique. Mutation gives rise to Lynch syndrome, probably of lower penetrance than in germline MLH1 and MSH2 mutations. Previously thought to be very rare in Lynch syndrome primarily due to technical difficulties in analysis. Mutations have recently been found to occur frequently using newly developed detection methods.

Somatic mutations

Probably very rare, putative somatic mutation reported. Supposedly last mismatch repair gene to undergo methylation.

POLE (*polymerase (DNA directed), epsilon, catalytic subunit*)**Location**

12q24.3

DNA / RNA

19 exons.

Protein

Member of the Family B DNA polymerases consisting of 4 subunits. Participates in chromosomal DNA replication, recombination as well as DNA repair via base excision and nuclear excision.

Germinal mutations

Sequence alterations, mainly in the catalytic subunit including the proofreading domain have been found in MLH1-mutant CRC. It has been suggested that defective polymerase proofreading can contribute to the mutator phenotype as a "booster" in CRC.

WNT-Signaling**APC (adenomatous polyposis coli)****Location**

5q21-q22

DNA / RNA

16 exons, 10702 bp mRNA.

Protein

Tumor suppressor protein composed of 2843 amino acids; the APC gene product interacts with the adherens junction proteins α - and β -catenin suggesting involvement in cell adhesion. APC may also inhibit the pathway regulated by the beta-catenin/Tcf complex.

Other functions include anterior-posterior pattern formation, axis specification, cell cycle, cell migration, apoptosis, chromosome segregation and spindle assembly.

Germinal mutations

Point mutations in APC gene results in the generation of stop codons, small deletions, LOH, 1-2 bp insertions. Most mutations result in a truncated protein, mostly in the first half. Results in FAP and AFAP.

Somatic mutations

The APC gene is mutated in about 50% of sporadic colorectal tumors. Most mutations are frameshifts or nonsense mutations resulting in premature stop codons. Some mutations in beta-catenin have been described in tumors and cell lines without mutations in the APC gene. APC loss may also be due to interstitial deletion or mitotic recombination, LOH, and nonsense mutations.

Most of the somatic mutations are clustered in the exon 15 in mutation cluster region (MCR).

About 20% of APC mutations are observed with mutation of TP53. Methylation of the promoter may be related.

DKK1 (dickkopf 1 homolog (*Xenopus laevis*))**Location**

10q11.2

DNA / RNA

12 exons, 3762 bp mRNA.

Protein

Human DKK1 is a potent inhibitor of WNT signaling over interaction with LRP5 and LRP6.

Somatic mutations

Genomic alterations (inactivation) are found in up to 33% of hypermutated CRC and 4% of non-hypermutated CRC cases.

LRP5 (low density lipoprotein receptor-related protein 5)**Location**

11q13.4

DNA / RNA

23 exons, 5161 bp mRNA.

Protein

LRP5 encodes a transmembrane low density lipoprotein receptor (1615 amino acids). It acts as a co-receptor with FZD10 on the cell surface, forming a Wnt-LRP5-LRP6-FZD10 complex for signal transduction of Wnt proteins.

Signal transduction is mediated by an intracellular PPP(S/T)P motif, which is phosphorylated following WNT activation.

The phosphorylated intracellular PPP(S/T)P motif then serves as a docking site for AXIN2.

Somatic mutations

Genomic alterations (inactivation) are infrequent in CRC and are found in <1% of hypermutated CRC and up to 10% of non-hypermutated cases.

FZD10 (frizzled family receptor 10)**Location**

12q24.33

DNA / RNA

1 exon, intronless, 3282 bp mRNA.

Protein

Encodes a 581 amino acid protein; member of the frizzled gene family seven-transmembrane-type WNT protein receptors. Acts as part of the Wnt-LRP5-LRP6-FZD10 receptor complex for signal transduction of WNT proteins. Upregulation leads to increased WNT signaling activity, inhibition of β -catenin degradation and accumulation of β -catenin in the cytoplasm.

Somatic mutations

Genomic alterations (activation) are found in 19% of non-hypermutated CRC and 13% of hypermutated CRC cases. Frequent events include overexpression of FZD10, in one series in up to 17% of cases.

AXIN1 (axin 1)**Location**

16p13.3

DNA / RNA

10 exons, 3675 bp mRNA.

Protein

Encodes an 862 amino acid protein. AXIN1 is an

important component of the beta-catenin destruction complex binding GSK3B, FAM123B and CTNNB1 (β -catenin). AXIN stabilizes this complex, which supports the hyperphosphorylation of β -catenin by GSK3B, followed by ubiquitination and degradation. AXIN thereby negatively regulates Wnt signaling; consequently loss of AXIN expression would be predicted to lead to decreased phosphorylation and degradation of β -catenin, causing increased WNT-signaling.

Somatic mutations

Limited data available. AXIN1 mutations (frequently missense mutations) have been described in up to 11% of colorectal cancer cases.

AXIN2 (axin 2)

Location

17q23-q24

DNA / RNA

10 exons, 4259 bp mRNA.

Protein

Encodes an 843 amino acid protein. Part of the axin protein family; Substitutes for AXIN1 as an important component of the beta-catenin destruction complex binding GSK3B, FAM123B and CTNNB1 (β -catenin).

Germinal mutations

Limited evidence suggests that germline mutations in AXIN2 may increase the risk for development of CRC.

Somatic mutations

Axin2 is frequently mutated in MMR deficient CRC cells. The mutations result in the stabilization of beta catenin. Genomic alterations are infrequent in non-hypermuted CRC. In hypermutated CRC up to 23% of cases show inactivation of the AXIN2 locus.

FAM123B (family with sequence similarity 123B)

Location

Xq11.2

DNA / RNA

2 exons, 7500 bp mRNA.

Protein

Encodes an 1135 amino acid protein. Forms the β -catenin destruction complex together with CTNNB1, GSK3B, AXIN1/AXIN2. Mutation is thought to contribute to the stabilization of β -catenin in CRC. Interacts with WT1.

Somatic mutations

The FAM123B gene contains a T6-microsatellite in the N-terminal coding region and is infrequently mutated in the setting of mismatch repair deficiency due to frame shift mutations (described in 5% of MSI cases in one series). More recent studies by the Cancer Genome Atlas Network have indicated genomic alterations (inactivation) in approximately 7% of non-hypermuted CRC and 37% of hypermutated CRC

cases. Further, FAM123B shows inactivating mutations in approximately 30% of Wilms tumors.

CTNNB1 (catenin (cadherin-associated protein), beta 1, 88kDa)

Location

3p21

DNA / RNA

16 exons, 3362 bp mRNA.

Protein

Encodes the 781 amino acid protein β -catenin. β -catenin functions as partner of E-cadherin in the formation of adherens junctions. Further, it is a central target of Wnt-signaling: In absence of Wnt-signaling, β -catenin forms a complex with APC, GSK-3 and Axin; Complex formation leads to phosphorylation of β -catenin through the action of GSK-3, which leads to degradation of the protein through the ubiquitin system. In case of APC mutation, β -catenin accumulates in the cell cytoplasm and translocates into the nucleus, where it has been shown to associate with transcription factors of the T cell factor-lymphoid enhancer factor (Tcf-Lef) family. This is assumed to lead to activation of genes involved in the positive regulation of epithelial to mesenchymal transition, proliferation and inhibition of apoptosis.

Germinal mutations

Germline inactivating mutations of the APC gene lead to the FAP syndrome.

Somatic mutations

Inactivating mutations in the APC gene represent early events in the chromosomal instability pathway of CRC. APC mutation leads to dissociation of the β -catenin-APC-GSK-3-Axin complex, and reduced phosphorylation and degradation of β -catenin. Genomic alterations (inactivation) are frequent in CRC, and are mostly due to biallelic inactivation of the APC gene, found in approximately 80% of non-hypermuted CRC and 50% of hypermutated CRC cases.

TCF7L2 (transcription factor 7-like 2 (T-cell specific, HMG-box))

Location

10q25.2

DNA / RNA

17 exons, predicted mRNA: 1490 bp.

Protein

Encodes a 619 amino acid transcription factor containing a high mobility group box; Interacts with CTNNB1 in the Wnt signaling pathway; Acts on numerous Wnt targeted genes containing a TCF7L2 binding domain.

Somatic mutations

In colorectal cancer cell lines, VTI1A-TCF7L2 fusions have been identified, supporting anchorage independent tumor growth. In CRC, genomic alterations

(inactivation) are found in approximately 12% of non-hypermethylated CRC and 30% of hypermethylated CRC cases.

FBXW7 (F-box and WD repeat domain containing 7, E3 ubiquitin protein ligase)

Location

4q31.3

DNA / RNA

10 exons, 3927 bp mRNA.

Protein

Encodes a 707 amino acid protein representing the variable F-box subunit of the SCF (SKP1-cullin-F-box) complex which functions as an ubiquitin ligase. FBXW7 acts as a tumor suppressor and is involved in the ubiquitination and degradation of the cell cycle regulators Cyclin E and c-Myc; further targets include notch intracellular domain (NICD), c-Myb, NOTCH1 and presenilin 1. Experimentally, loss of FBXW7 leads to accumulation of β -catenin; Further, mutation of FBXW7 has been suggested to increase cell proliferation.

Germinal mutations

Somatic mutations of FBXW7 in HNPCC and FAP patients have been described. The frequency of FBXW7 mutations in FAP and HNPCC corresponded to patients with sporadic carcinomas.

Somatic mutations

Somatic mutations have been described in ovarian and breast cancer cell lines. In CRC, genomic alterations (inactivation) are found in approximately 10% of non-hypermethylated CRC and 43% of hypermethylated CRC cases.

SOX9 (SRY (sex determining region Y)-box 9)

Location

17q23

DNA / RNA

3 exons, 3963 bp mRNA.

Protein

Encodes a 509 amino acid protein which contains a high-mobility-group (HMG) domain; Acts as a transcription factor and inhibits CTNNB1 (β -catenin) activity; In particular, the SOX9 product has been shown to interfere with the activation of β -catenin dependent gene targets and acts as a promoter β -catenin ubiquitinylation and degradation; important in skeletal development.

Somatic mutations

In CRC, genomic alterations of SOX9 are infrequently described. Genomic alterations are found in

approximately 4% of non-hypermethylated CRC and 7% of hypermethylated CRC cases.

TGF β -Signaling

TGFBR1 (transforming growth factor, beta receptor 1)

Location

9q22

DNA / RNA

9 exons, complex locus; transcription produces 11 different mRNAs.

Protein

The 503 amino acid TGFBR1 proteins form a homodimer and associate with TGFBR2 homodimers to form the TGF-beta receptor; Both subunits of the TGF-beta receptor, TGFBR1 and TGFBR2, act as transmembrane serine/threonine kinases. After binding of the TGF-beta cytokine dimers TGF β 1, TGF β 2 or TGF β 3, the TGFBR2 and TGFBR1 form a stable receptor complex; TGFBR2 is a constitutively active type II transmembrane serine/threonine kinase and phosphorylates TGFBR1 (a type I receptor) upon ligand binding. The TGFBR1 serine/threonine kinase binds and phosphorylates SMAD2, a receptor-associated signaling molecule. SMAD2 dissociates from the receptor and interacts with SMAD4; the SMAD2/SMAD4 complex then translocates into the nucleus and modifies the transcription of TGF-beta regulated genes. TGF-beta mediated signaling has been shown to have anti-proliferative effects on epithelia, mesenchymal and hematopoietic cells and to modulate cell-stroma interactions. Physiologic TGF-beta signaling promotes cell differentiation and can induce apoptosis. In cancer cells, TGF-beta signaling is frequently disrupted by mutations in the receptor or downstream mediators turning off the signaling pathway; In the tumor microenvironment, TGF-beta modulates tumor-host interaction by increasing angiogenesis and converting T-effector cells into immunosuppressive Regulatory T-cells, thereby supporting tumor progression and inhibiting the anti-tumoral immune defense. In conjunction with IL-6, TGF-beta has been shown to induce the differentiation of Th17 cells.

Germinal mutations

An increased prevalence of SNPs in the TGFBR1 locus resulting in decreased TGFBR1 allelic expression (up to 9.3% of cases) have been shown in patients with colorectal cancer.

Somatic mutations

In CRC, genomic alterations (inactivation) of TGFBR1 are frequent in patients with hypermethylated CRC (17%). Genomic alterations of the TGFBR1 locus are infrequently observed in patients with non-hypermethylated CRC (<1%).

TGFBR2 (transforming growth factor, beta receptor II (70/80kDa))**Location**

3p22

DNA / RNA

8 exons, 2094 bp mRNA.

Protein

Encodes a 567 amino acid protein which forms a homodimer and associates with TGFBR1 to form the TGF-beta type II receptor.

Possesses serine/threonine kinase function and activates SMAD2 and SMAD3 by phosphorylation. Activated SMAD2 forms a heterodimer with SMAD4 to form a complex which translocates into the nucleus and modulates the transcription of TGF-beta regulated genes.

Somatic mutations

The TGFBR2 gene locus contains a poly A mononucleotide tract in the coding region, which is frequently mutated in MSI-high CRC (up to 74%), leading to inactivation of the gene.

TGFBR2 mononucleotide mutations have not shown an impact on outcome in initial studies. Inactivation of TGFBR2 by point mutation has been demonstrated in up to 15% of MSS CRC cases in initial studies.

More recent studies by the Cancer Genome Atlas Network have indicated genomic alterations (inactivation) in approximately 2% of non-hypermuted CRC and 43% of hypermutated CRC cases.

SMAD2 (SMAD family member 2)**Location**

18q21.1

DNA / RNA

12 exons, 10384 bp mRNA.

Protein

Encodes a 467 amino acid protein; Downstream signaling molecule of TGF-Beta signaling. SMAD2 is phosphorylated by the heteromeric TGF-beta type II receptor serine/threonine kinase domain. SMAD2 forms a dimer with SMAD4 followed by translocation into the nucleus; the SMAD2/SMAD4 dimer then modifies the transcription of TGF-Beta target genes by forming a transcription repressor complex.

Somatic mutations

Somatic mutations of SMAD2 have been described in 3.4% of sporadic CRC, mostly causing missense changes in the protein which reduces SMAD2/SMAD4 complex formation. Joint biallelic hits are common.

Recent studies by the Cancer Genome Atlas Network indicate inactivating genomic alterations in 6% of non-hypermuted CRC and 13% in hypermutated cases.

SMAD3 (SMAD family member 3)**Location**

15q22.33

DNA / RNA

9 exons, 5808 bp mRNA.

Protein

Encodes a 425 amino acid protein; Downstream signaling molecule of TGF-Beta and activin signaling. Phosphorylated by the heteromeric TGF-beta type II receptor serine/threonine kinase and the activin type I receptor kinase domain. SMAD3 forms a dimer with SMAD4 followed by translocation into the nucleus; the SMAD3/SMAD4 dimer then modifies the transcription of TGF-Beta target genes.

Somatic mutations

Somatic mutations of SMAD3 have been described in 4.3% of sporadic CRC. Missense changes in the protein lead to reduced association with SMAD4 by alteration of the phosphorylation regulated Ser-Ser-X-Ser motifs within SMAD3. Further, mutations cause reduced SMAD3 transcriptional activity. Joint biallelic hits are common. Recent data from the Cancer Genome Atlas Network indicate infrequent genomic alterations in 2% of non-hypermuted CRC. Inactivating genomic alterations are described in up to 17% of hypermutated cases.

SMAD4 (SMAD family member 4)**Location**

18q21.1

DNA / RNA

13 exons, 3202 bp mRNA.

Protein

The SMAD4 protein is coded from 11 exons and contains 552 amino acids. SMAD4 forms a heterodimer with phosphorylated SMAD2 or SMAD3 and translocates into the nucleus. The SMAD2/SMAD3-SMAD4 complex then forms a transcription repressor complex and modifies transcription of TGF-Beta target genes.

Germinal mutations

Results in juvenile polyposis with an approximate lifetime risk of 40% for development of CRC.

Somatic mutations

Somatic mutations of SMAD4 have been described in 8.6% of sporadic CRC. Missense changes in the protein structure reducing the association with SMAD2 in the SMAD2/4 complex. Joint biallelic hits are common. Recent studies by the Cancer Genome Atlas Network indicate inactivating genomic alterations in 15% of non-hypermuted CRC and 20% in hypermutated cases.

ACVR2A (activin A receptor, type IIA)**Location**

2q22.3

DNA / RNA

11 exons, 5217 bp mRNA.

Protein

The 513 amino acid ACVR2A proteins forms a homodimer (type IIA receptor subunit) and associate with ACVR1B homodimers (type IB receptor subunit) to form the Activin receptor; Both ACVR2A and ACVR1B act as transmembrane serine/threonine kinases. After binding of activin A, activin B or inhibin A, which belong to the TGF-Beta protein superfamily, ACVR2A and ACVR1B form a stable receptor complex; ACVR2A is a type II transmembrane serine/threonine kinase and phosphorylates ACVR1B (a type I receptor) upon ligand binding. The ACVR1B serine/threonine kinase binds and phosphorylates receptor-associated signaling molecule from the SMAD family (SMAD2, SMAD3). SMAD2 dissociates from the receptor and interacts with SMAD4; the SMAD2/SMAD4 complex then translocates into the nucleus and modifies the transcription of TGF-beta regulated genes.

Somatic mutations

Somatic mutations in ACVR2A are particularly common in MSI-high CRC. The ACVR2A locus encompasses two poly A repeats within the coding sequence of exon 3s and 10 which have been shown to be mutated in up to 83% of MSI-high CRC leading to loss of protein expression in up to 62% of the cases with mutations. Further studies have identified methylation and LOH of the ACVR2A locus in association with loss of protein expression.

ACVR1B (activin A receptor, type IB)**Location**

12q13

DNA / RNA

7 exons, 2375 bp mRNA.

Protein

The 505 amino acid ACVR1B proteins (type IB receptor subunit) form the activin receptor complex in association with ACVR2A or ACVR2B homodimers (type IIA receptor subunit) to form the Activin receptor; Act as transmembrane serine/threonine kinases and activate receptor-associated signaling molecule from the SMAD family (SMAD2, SMAD3).

Somatic mutations

Data on genomic alterations in CRC are scarce. Recent studies by the Cancer Genome Atlas Network indicate frequent inactivating genomic alterations in 20% of hypermutated cases. Genomic alterations of the ACVR1B locus are infrequently observed in patients

with non-hypermutated CRC (4%). Frequent somatic mutations have been described in pancreatic cancer (up to 30%).

RTK-RAS-Signaling**ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian))****Location**

17q12

DNA / RNA

30 exons, 4478 bp.

Protein

The Epidermal Growth Factor Receptor (EGFR) family consists of 4 receptor tyrosine kinases, EGFR (ERBB1/HER1), ERBB2 (HER2), ERBB3 (HER3) and ERBB4 (HER4). ERBB2 encodes a 1255 aa protein that has no ligand binding domain of its own and therefore cannot bind growth factors. However, it does bind tightly to other ligand-bound EGFR family members to form a heterodimer, stabilizing ligand binding and enhancing kinase-mediated activation of downstream signaling pathways.

Somatic mutations

Mutations and focal amplifications of ERBB2 in colorectal cancers are rare (<5%). As potentially drug-targetable, the predictive value of ERBB2 amplifications in these patients remains to be determined.

ERBB3 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian))**Location**

12q13

DNA / RNA

28 exons, 5511 bp.

Protein

1342 aa. ERBB3 possesses an extracellular ligand-binding domain, a transmembrane domain and a tyrosine kinase domain. However, the kinase domain has no tyrosine kinase activity. Erbb3 therefore can bind ligand but cannot convey the signal into the cell through protein phosphorylation. It however does dimerize with other EGF receptor family members.

Somatic mutations

Mutations in ERBB3 appear only as rare events in colorectal cancers.

KRAS (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog)**Location**

12p12.1

DNA / RNA

6 exons, 5436 bp.

Protein

189 aa. KRAS belongs to the ras gene family containing also HRAS and NRAS. The KRAS protein is a GTPase that plays a central role in several different signal transduction pathways. In colorectal cancer, ligand binding and heterodimerization of the EGFR leads to activation of the oncogenic Ras-Raf-MEK-ERK signaling pathway acting on cell proliferation, cell survival and tumor angiogenesis.

Somatic mutations

Activating point mutations in KRAS occur in approximately 30-40% of all patients with colorectal cancer and are frequent in exon 2 codons 12 and 13 as well as in exon 3 codon 61. These mutations are no longer considered to be of prognostic value. Evidence suggests that metastatic patients with KRAS mutated colorectal cancers undergoing anti-EGFR based therapies have a significantly reduced treatment response rate compared to patients with wild-type tumors. Nonetheless, recent retrospective analyses indicate that specific KRAS gene mutations, for example, G13D, may confer a degree of responsiveness in these patients.

NRAS (neuroblastoma RAS viral (v-ras) oncogene homolog)**Location**

1p13.2

DNA / RNA

7 exons, 4461 bp.

Protein

189 aa. N-ras belongs to the ras gene family containing also KRAS and HRAS. NRAS is an oncogene that encodes a membrane protein with GTPase activity that is activated by a guanine nucleotide-exchange factor and inactivated by a GTPase activating protein.

Somatic mutations

Substitution missense mutations occurring predominantly in codons 12, 13 and 61 have been reported in <5% of patients with colorectal cancer. These mutations may have predictive value as biomarkers of non-response in patients treated with anti-EGFR based therapies.

BRAF (v-raf murine sarcoma viral oncogene homolog B1)**Location**

7q34

DNA / RNA

18 exons, 2949 bp.

Protein

766 aa. Braf is an oncogenic protein and one of three Raf kinases that play a role in the Ras-Raf-MEK-ERK signaling pathway by phosphorylating MEK.

Germinal mutations

BRAF mutations generally do not occur in patients with Lynch syndrome associated colorectal cancers (HNPCC) and are often used as an exclusion criterion for detection of these patients.

Somatic mutations

BRAF point mutations in colorectal cancers occur in 9-12% of all patients. They are commonest in exon 15 codon 600 (p.V600E).

BRAF gene mutations are frequently detected in patients with microsatellite instability-high (MSI-H) colorectal cancers as well as in tumors displaying high-level CpG Island Methylator Phenotype (CIMP). BRAF mutations have been linked to more unfavorable survival, often independently of the MSI status; they are not predictive of response in patients treated with anti-EGFR based therapies.

PI3K-Signaling**IGF2 (insulin-like growth factor 2 (somatomedin A))****Location**

11p15.5

DNA / RNA

5 exons, 1354 bp mRNA.

Protein

Encodes a 180 amino acid protein; member of the family of insulin-like polypeptide growth factors recognized by the Insulin like growth-factor 1 receptor (IGF1R).

Activation of IGF1R (a receptor tyrosine kinase) leads to phosphorylation of insulin-receptor substrates 1 and 2 (IRS1/IRS2), which contribute to the activation of both the PI3K-AKT/PKB pathway and the Ras-MAPK pathway.

PI3K-AKT/PKB regulates cell survival and sensitivity to apoptosis; Ras-MAPK signaling controls cell proliferation, cell survival and tumor angiogenesis.

Both pathways play important roles for malignant transformation and tumor progression in CRC.

Somatic mutations

15-22% of colorectal cancers show overexpression of IGF2, which is particularly common in non hypermutated, MSS CRC. IGF2 overexpression has not been described as a pathogenetic feature of MSI-high CRC.

PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha)**Location**

3q26.3

DNA / RNA

21 exons, 3207 bp mRNA.

Protein

Encodes a protein predicted with 1068 amino acids,

representing the catalytic subunit alpha of class I PI 3-kinases (PI3K). PI3K are a group of enzymes that play a central function for intracellular signal transduction by the generation of phosphatidylinositol 3,4,5-trisphosphate at the plasma membrane. PI3K generates PI3 by phosphorylating phosphatidylinositol 4,5-diphosphates (PI2) in the plasma membrane which then serve as docking sites for proteins containing a phosphoinositide-binding domains (e.g. pleckstrin homology domains). This includes protein kinase B (Akt), thereby triggering the Akt/mTOR pathway. The Akt pathway has a central regulatory role and pleiotropic effects on cell growth, differentiation and proliferation, cell motility and migration as well as the regulation of angiogenesis and apoptosis.

Somatic mutations

In CRC, activating mutations are frequently observed in exon 9 and exon 20 of the PIK3CA locus. Frequency of genomic alterations ranges from approximately 15% in non-hypermuted CRC to 37% in hypermutated cases. PIK3CA exon 20 mutations have been correlated to a reduced response rate of CRC patients to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies. Further, concomitant exon 9 and exon 20 mutations have been shown to predict significantly worse disease-specific survival while isolated mutations in either exon 9 or 20 did not impact survival outcome.

PTEN (phosphatase and tensin homolog)

Location

10q23.3

DNA / RNA

9 exons, 3147 bp mRNA.

Protein

Tumor suppressor protein, which negatively regulates AKT/PKB signaling. The protein is a dual specificity phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase, removing phosphates from serine, tyrosine and threonine; Further, PTEN acts as a lipid phosphatase, removing phosphate groups from phosphatidylinositol 3,4,5-trisphosphate, thereby antagonizing PI 3-kinase function and negatively regulating the Akt/mTOR pathway; PTEN thereby assumes tumor-suppressor functions, inhibiting cell growth, proliferation, motility and migration and supporting apoptosis in many different cancer subtypes.

Germinal mutations

Germinal mutations in PTEN are associated with the hereditary Cowden Syndrome which is characterized by development of multiple hamartomas in skin and gastrointestinal tract (intestinal hamartomatous polyps), gangliocytoma of the cerebellum and a severely increased risk for the development of breast carcinoma, non-medullary carcinoma of the thyroid, endometrial

carcinoma, colorectal cancer, kidney cancer and melanoma.

Further, germinal mutations in PTEN are associated with rare hereditary syndromes including Bannayan-Riley-Ruvalcaba Syndrome, Proteus syndrome and Proteus-like syndrome.

Somatic mutations

Somatic mutations of PTEN are more frequently observed in MSI-high CRC (18% of cases) than MSS CRC. Somatic mutations (inactivation) in MSI-high CRC are the consequence of frameshift mutations, nonsense, missense, and splice-site mutations.

P53-Signaling

TP53 (tumor protein p53)

Location

17p13

DNA / RNA

11 exons, 1179 bp mRNA.

Protein

Encodes a 393 amino acid protein. P53, the "master watchman of the genome" has central tumor suppressor function and an essential role in cell cycle regulation, particularly at the G1 DNA damage checkpoint. The five highly-conserved gene regions contain a transactivation domain, a DNA-binding domain, nuclear localization signals and a tetramerization domain. Intracellular P53 levels are low in cellular homeostasis, but are quickly upregulated in response to cellular and oncogenic stress, in particular DNA damage. P53 targets genes involved in the cell cycle, stopping the progression of the G1 phase (through activation of p21) if DNA damage or other stress signals are present.

Germinal mutations

Results in the autosomal dominant Li-Fraumeni Syndrome, which leads to a severely increased risk for the development of soft tissue tumors (sarcomas, osteosarcomas), brain tumors (glioblastoma) as well as hematologic malignancies (leukemia) and epithelial malignancy (breast cancer, adrenal cortical carcinoma). An increased risk for colorectal carcinomas has been described in initial studies, mostly in association with missense or nonsense mutations between exons 4-10.

Somatic mutations

Mutations of P53 are most frequent in non-hypermuted cancers (59%), and are mostly located in exons 4 to 8 with hotspots at codons 175, 245, 248, 273 and 282.

Both deletions and insertions as well as missense, nonsense and splicing mutations have been described resulting in a truncated p53 protein. 80% of mutations result from deamination of methylated cytosine in CpG region of the gene (codons 175, 248 and 273).

Mutant p53 is found to be overexpressed in primary colon cancer. p53 mutation is observed in 40-50% of

colorectal carcinomas, and is associated with aggressive carcinomas. p53 mutation or LOH of chromosome 17p is observed mostly in carcinoma rather than adenoma, in both familial and non-familial patients.

Others

ARID1A (AT rich interactive domain 1A (SWI-like))

Location

1p35.3

DNA / RNA

20 exons, 8585 bp mRNA.

Protein

Encodes the 2285 amino acid AT-rich interactive domain-containing protein 1A (ARID1A); member of the SWI/SNF family; ARID1A possesses helicase and ATPase function and regulates transcriptional activation and repression of target genes through modification of the chromatin structure, including the MYC-oncogene (repression).

Somatic mutations

Somatic (truncating) mutations have been described in up to 10% of colorectal cancers, with more than half of these cases found to be associated with microsatellite instability (out-of-frame insertions or deletions at mononucleotide repeats).

Recurrent inactivating genomic alterations have been confirmed in up to 37% of hypermutated cases in recent studies by the Cancer Genome Atlas Network; genomic alterations non-hypermutated CRC were infrequent (5%).

STK11 (serine/threonine kinase 11)

Location

19p13.3

DNA / RNA

10 exons, 3276 bp mRNA.

Protein

Encodes a 433 amino acid protein.

Tumor suppressor serine-threonine kinase, with important functions in the activation of adenine monophosphate-activated protein kinase (AMP)-activated protein kinase kinases in the setting of energy deprivation; Activation of AMP kinases contributes to the maintenance of cell polarity and negatively regulates cell cycle progression and cell growth.

Germinal mutations

Mutations (Nonsense, missense, frameshift), result in the autosomal dominant Peutz-Jeghers syndrome, which results in the formation of benign hamartomatous polyps in the gastrointestinal tract; other characteristics include pigmented macules on the oral mucosa and lips.

Patients with Peutz-Jeghers syndrome carry a severely increased risk for the development of CRC estimated at about 39%.

Somatic mutations

Somatic mutations in STK11 are rare in sporadic colorectal cancer outside inherited hamartomatous syndromes.

BMPR1A (bone morphogenetic protein receptor, type IA)

Location

10q22.3

DNA / RNA

13 exons, 3616 bp mRNA.

Protein

Encodes a 532 amino acid protein; Transmembrane serine-threonine kinase, type1 receptor, closely related to the ACVR1 and ACVR2 activin receptors; ligands include bone morphogenetic protein 2 and 4 (BMP-2 and BMP-4) from the TGF-Beta superfamily.

Germinal mutations

Nonsense, missense, frameshift and splice-site mutations.

Associated with Juvenile polyposis, which carries an approximate lifetime risk of 40% for development of CRC.

MUTYH (mutY homolog (E. coli))

Location

1p34.1

DNA / RNA

16 exons, 1839 bp mRNA.

Protein

DNA glycosylase.

Functions in oxidative DNA damage repair (base excision repair), nicks A-G mismatches, as well as A-8oxoG and A-C mismatches.

Germinal mutations

Tyr82-Cys and Gly253 -Asp transitions affect glycosylase activity, resulting in APC mutations in somatic cells. Nonsense mutations, missense and truncated protein mutations. Mutations cause 93-fold increase in colorectal cancer risk.

DCC (deleted in colorectal carcinoma)

Location

18q21.3

DNA / RNA

29 exons, 4609 bp mRNA.

Protein

DCC is thought to be receptor for neptin-1 (axonal chemoattractant). In absence of ligand, it induces apoptosis but when neptin-1 is bound, it prevents apoptosis.

Germinal mutations

Chromosome 18 sequences are frequently (74%) lost in colorectal carcinoma. Loss of DCC is mostly observed in metastatic cancers.

To be noted

Note

The RER+ sporadic colon cancers are mostly diploid, without LOH, with few mutations of p53 and APC and right-sided; they contain mutations in repetitive coding sequences of a number of genes such as the TGFbeta type II receptor, the receptor of the Insulin-like growth factor and the BAX gene implicated in apoptosis.

The RER- are polyploid, with LOH (5q, 17p, 18q), mutations in p53, and more often left-sided, they have a worse prognosis.

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