Molecular genetics of colorectal cancer

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Abstract

Approximately 90% of colorectal cancer cases are sporadic without family history or genetic predisposition, while in less than 10% a causative genetic event has been identified. Historically, colorectal cancer classification was only based on clinical and pathological features. Many efforts have been made to discover the genetic and molecular features of colorectal cancer, and there is more and more evidence that these features determine the prognosis and response to (targeted) treatment. Colorectal cancer is a heterogeneous disease, with three known major molecular groups. The most common is the chromosomal unstable group, characterized by an accumulation of mutations in specific oncogenes and tumor suppressor genes. The second is the microsatellite unstable group, caused by dysfunction of DNA mismatch repair genes leading to genetic hypermutability. The CpG Island Methylation phenotype is the third group, distinguished by hypermethylation. Colorectal cancer subtyping has also been addressed using genome-wide gene expression profiling in large patient cohorts and recently several molecular classification systems have been proposed. In this review we would like to provide an up-to-date overview of the genetic aspects of colorectal cancer.

Keywords Colorectal cancer, molecular pathways, genetics, subtypes

Introduction

With more than 1.2 million new cases diagnosed annually, colorectal cancer (CRC) is the third most common cancer worldwide [1] and is therefore considered a major health problem.

Less than 10% of patients with CRC have a true inherited predisposition to CRC. In most of these cases, the causative genetic event has been identified. However, up to 25% of cases have a family history of CRC (familial CRC), but are not consistent with one of the known inherited syndromes. They have a higher risk of developing CRC in comparison with the general population, although not as high as in the inherited syndromes. Most of the CRC cases however are sporadic, in which there is no family history or genetic predisposition.

Hereditary colon cancer

Familial adenomatous polyposis (FAP)

FAP is characterized by hundreds to thousands of adenomatous colorectal polyps that develop in the second decade of life. It accounts for approximately 1% of CRC cases. FAP has an incidence of 1/10,000 – 30,000 and manifests equally in both men and women [4]. If not identified and treated at an early stage, there is a 100% risk of developing CRC by the age of 40, with colon cancer occurring 10 years after the onset of the polyps. FAP is inherited in an autosomal dominant manner by a germline mutation in the adenomatous polyposis coli (APC) gene. Most patients have a family history of the disease, however approximately 25% emerge as ‘de novo’ gene mutations in the APC gene [5].
More than 1000 different mutations of the APC gene are described as a cause of FAP [6]. These mutations (e.g. insertion, deletion, nonsense mutation), result in a truncated APC protein. In normal individuals, the APC tumor suppressor protein plays a central role in the Wnt signaling pathway by regulating the degradation of β-catenin. β-Catenin acts as a transcription factor for proliferation genes. Accumulation of the oncogenic protein β-catenin is prevented by the APC-gene product, thereby controlling proliferation of the intestinal crypt epithelial cells. Mutation in the APC gene leads to loss of APC function and results in an accumulation of β-catenin. To develop cancer, a mutation of APC must be followed by other mutations.

90% of FAP patients develop upper gastrointestinal tract polyps including fundic gland polyps in the stomach, duodenal and periampullary adenomatous polyps [7]. About 5% of the duodenal polyps progress to cancer within 10 years and it is the second cause of death in FAP patients [8]. FAP can present with extra-intestinal manifestations such as osteomas, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium (CHRPE), desmoid tumors and extracolonic cancers (thyroid, bile duct, liver, central nervous system).

Attenuated FAP (AFAP) is a less aggressive variant of FAP, characterized by fewer (10-100) adenomatous polyps that appear at a later age and have a lower cancer risk [9]. Polyps are mainly found in the proximal colon and infrequently in the rectum.

The main goals in the management of patients with FAP are cancer prevention and maintaining a good quality of life. Around the age of 16 years, annual colonoscopy is recommended and all adenomas of significant size should be removed. Due to the increasing number of adenomas, prophylactic cancer-preventive colorectal surgery is necessary by the age of 20. Even after colectomy follow up is vital to detect adenomatous polyps of the remaining gastrointestinal tract, and to identify desmoid tumors in their earliest stage.

MUTYH-associated polyposis (MAP)

A subset of patients with clinical FAP and AFAP, without a strong multigenerational family history, does not have a detectable APC gene mutation. In these patients an autosomal recessive disorder, MAP, is frequently seen [6]. This condition is caused by a biallelic germline mutation in the base-excision-repair (BER) gene MUTYH. About 30% of patients will also develop polyps in the upper gastrointestinal tract, but no extra-intestinal manifestations are seen [4]. Patients have an 80% risk of developing CRC and the mean age of diagnosis is between 40 and 60 years old [10]. When diagnosed, the management is similar to those with FAP.

Peutz-Jeghers syndrome (PJS)

PJS is a very rare autosomal dominant genetic disorder, characterized by multiple hamartomatous polyps of the gastrointestinal tract, most often found in the small intestine. The polyps are 0.1-5 cm in diameter and the number varies between 1 and 20 per segment of the gastrointestinal tract [11]. The most characteristic extra-intestinal manifestations are mucocutaneous lesions causing patches of hyperpigmentation in the mouth and on the hands and feet, which usually occur in infancy and fade in late adolescence. Patients with PJS have a germline mutation of the serine threonine kinase 11 (STK-11), a tumor suppressor gene. Adults with PJS not only have a high risk of developing gastrointestinal cancer, but also non-gastrointestinal cancers, especially breast cancer.

Serrated polyposis syndrome (SPS)

SPS, formerly known as the hyperplastic polyposis syndrome, is a relatively rare syndrome characterized by multiple serrated polyps of the colon. A patient is diagnosed with SPS if at least one of the following criteria is present: 1) at least 5 serrated polyps proximal to sigmoid, two of which >10 mm; 2) serrated polyps proximal to sigmoid in an individual who has a first-degree relative with SPS; and 3) >20 serrated polyps (any size) distributed throughout the colon [12]. At first, hyperplastic polyps were considered to be non-neoplastic lesions, until 1996 when Torlakovic and Snover demonstrated histological differences between the polyps in SPS and sporadic hyperplastic polyps [13]. Moreover, SPS has been associated with an increased incidence of CRC [14]. Subsequently, hyperplastic polyps have been renamed as serrated polyps and WHO distinguishes three categories of serrated polyps: hyperplastic polyps, sessile serrated adenomas and traditional serrated adenomas [15]. The genetic basis of SPS remains unknown, both recessive and dominant transmission has been proposed. Most likely, there exists more than one genetic cause of SPS.

Hereditary non-polyposis colorectal cancer (HNPCC)

HNPCC or Lynch syndrome is the most common inherited colon cancer syndrome, caused by a germline mutation in one of several mismatch repair (MMR) genes. It is an autosomal dominant genetic condition, characterized by increased risk for CRC and endometrial cancer as well as smaller risk of some other cancers (ovary, gastric, small intestine, hepatobiliary tract, upper urinary tract, brain and skin) [16]. About 2-5% of all CRC cases are attributed to HNPCC. The combination of a germline mutation in an MMR gene with inactivation of the remaining normal allele, results in loss of MMR function and accumulation of mutations in microsatellites. HNPCC defects in DNA MMR genes leads to microsatellite instability (MSI), a hallmark of HNPCC.

Sporadic colon cancer

Thanks to the genetic revolution, major progress has been made in understanding the molecular basis of sporadic colon cancer. In 1990, Vogelstein described in his multistep genetic model that the accumulation of multiple genetic mutations
led to a selective growth advantage to the epithelial cells in the colon [17]. Furthermore he described that the total accumulation of genetic alterations, rather than their order, is responsible for the biological behavior. At that time, colon cancers were believed to progress through an adenoma - carcinoma sequence which still holds true for the majority of CRCs that arise from premalignant polyps. In the Vogelstein model, APC mutations serve as the initiating event in adenoma formation followed by accumulation of multiple mutations. Although according to the Vogelstein model at least 7 distinct mutations are required for tumorigenesis, genome-wide sequencing of colon cancers has calculated about 80 mutated genes per colorectal tumor, however less than 15 mutations were considered to be true drivers [18]. Recently the existence of alternative routes of colon cancer carcinogenesis through serrated polyps has been described [15].

At least three major distinct molecular pathways have been described, leading to CRC. The first and most common (70%), the chromosomal instability (CIN) pathway, defines the accumulation of numerical (aneuploidy) or structural chromosomal abnormalities, resulting in karyotypic variability from cell to cell [19], and is characterized by frequent loss-of-heterozygosity (LOH) at tumor suppressor gene loci and chromosomal rearrangements. Moreover, CIN tumors are distinguished by the accumulation of mutations in specific oncogenes (e.g. APC, KRAS, PIK3CA, BRAF, SMAD4, TP53, etc.) and tumor suppressor genes, thereby activating pathways critical for CRC tumorigenesis. Whether CIN creates the appropriate environment for the accumulation of these mutations or vice versa remains unknown [20].

Another important pathway leading to genomic instability is the MSI pathway, caused by dysfunction of DNA MMR genes leading to genetic hypermutability. Microsatellites are nucleotide repeat sequences of 1-6 base pairs in length. These sequence motifs are prone to accumulation of mutations, mainly because DNA polymerases can’t bind DNA efficiently. Insertions and/or deletions in microsatellites located in DNA coding regions lead to frameshift mutations, which can lead to protein truncations. Deficiency in DNA repair capacity due to silencing of MMR genes gives rise to the accumulation of abnormalities in short sequences that are repeated up to hundreds of times within the genome (microsatellites). The phenotype is characterized by right-sided location, mucinous cell type and presence of tumor infiltrating lymphocytes. Besides being the hallmarks of HNPCC, MSI is also found in 15% of the sporadic CRC caused by an epigenetic phenomenon, that is hypermethylation of the gene promoter for the MMR enzyme (usually MLH1) leading to gene silencing [21]. The presence of widespread CpG island methylation in CRC, leads us to the third pathway, which is designated the CpG Island Methylation Pathway (CIMP) [22]. Most sporadic MSI colon tumors are CIMP positive and are usually located in the proximal site of the colon (up to 40%). Activating mutations in BRAF occur almost exclusively in MSI, CIMP positive CRC. It has been suggested that CIMP positive tumors can be divided in two types, namely CIMP high, related to BRAF mutations and MLH1 methylation and CIMP low, related to KRAS mutations [23]. Since the definition of the three pathways is not mutually exclusive, it is possible that tumors can exhibit features of multiple pathways.

In conclusion, although 70% of CRC arise via the well characterized chromosomal instability pathway, it seems that approximately 30 % of CRC develop via a serrated pathway which is characterized by activation of the MAPK pathway (KRAS or BRAF mutations, mutually exclusive) and the presence of CIMP (L or H) [15]. Although often present, MSI is not required for the serrated neoplasia pathway. This proves that the serrated pathway carcinomas are also still a very heterogeneous group. Recently, a preclinical progression model of BRAF-induced carcinogenesis has been proposed which progresses through a hyperplasia / adenoma / carcinoma sequence [24].

As it has become clear that CRC is not a single disease, but rather a heterogeneous complex of diseases, it is believed that CRC with similar characteristics most likely share the same pathogenesis and biological behavior. Historically, CRC classification was only based on clinical and pathological features. Adding molecular features is important because it reflects the mechanisms of carcinogenesis. Based on a link between pathological, molecular and clinical features Jass et al classified CRC in to 5 subtypes: 1) CIMP-H / MSI-H/ BRAF mutation; 2) CIMP-H / MSI-L or microsatellite stable (MSS)/ BRAF mutation; 3) CIMP-L / MSS or MSI-L / KRAS mutation; 4) CIMP-negative / MSS; and (5) HNPCC / CIMP-negative / MSI-H [25].

Growing evidence suggests that epigenetic changes might even be higher than the genetic changes and are a major determinant in the origin of the tumor and tumor heterogeneity. Aberrant DNA methylation of CpG islands has been reported in early lesions in colon mucosa [26]. As already stated, CRC with a CIMP phenotype, exhibit a high frequency of cancer-specific DNA hypermethylation and are highly enriched for BRAF mutations [21]. To better characterize DNA methylation subgroups in CRC, Hinoue et al performed genome-scale DNA methylation profiling of 125 primary colon tumors and 29 adjacent non-tumor colonic mucosa together with gene expression to assess the biological implications of DNA methylation-mediated gene silencing [27]. They identified four subgroups, with each subgroup showing characteristic genetic and clinical features, indicating that they represent biologically different subgroups. First, a CIMP-H subgroup which is strongly associated with the BRAF<sup>V600E</sup> mutation, second a CIMP-L subgroup enriched for KRAS mutations, third a non-CIMP group with a significantly higher frequency of TP53 mutations and located mainly in the distal colon and finally another non-CIMP group with a low frequency of gene mutations and enriched for rectal tumors.

In 2012, the Cancer Genome Atlas (TCGA) project published the result of full genomic profiling of 276 colorectal cancer samples, including exome sequencing, DNA copy number, promoter methylation, mRNA and miRNA expression [3]. The hypermutated CRC comprised 16% of total samples, of which three quarters were as expected MSI-H, usually with hypermethylation and MLH1 silencing, the other quarter were surprisingly not MSI, but showed somatic mutations in MMR genes and polymerase ε (POLE). In addition, the hypermutated
tumors were found predominantly in the right colon and mostly hypermethylated. Moreover, these hypermutated tumors are highly immunogenic because of the generation of mutated proteins (including frame-shift mutations) [28]. In the other non-hypermutated group, patterns of genomic similarity were found with overall 24 genes that were significantly mutated, including APC and TP53. Remarkably, these 2 genes were more frequently mutated in the non-hypermutated group than in the hypermutated ones, suggesting that CRC from both groups develop differently on a genetic level.

CRC subtyping has also been addressed using genomewide gene expression profiling in large patient cohorts [29-32]. Salazar et al performed unsupervised hierarchical clustering of 188 stage I to IV CRC and revealed three main molecular subtypes with different prognosis [29]. Another study identified four subgroups of which one correlated with MSI, BRAF mutations and mucinous histology [31]. Recently, Budinska et al characterized five main subtypes of CRC in terms of biological motifs, common clinical variables, association with known CRC molecular markers and morphological patterns [30]. They called their subtypes surface crypt like, lower crypt-like, CIMP-H like, mesenchymal and finally a mixed group. The authors concluded that their subtypes should be prospectively assessed for their clinical relevance.

Two recent studies published in *Nature Medicine* [33,34] also used gene expression profiling to classify CRC and to correlate with prognosis and response prediction. De Sousa et al defined three groups which correlated well with two of the known molecularly pathways, namely the CIN (hereby named CCS1) and the MSI (CCSA) group [33]. The third group overlaps partly with the CIMP group (CCS3), might be derived from the serrated pathway and is linked with poor prognosis. Sadanandam et al defined six groups that are related to six different cells of origin in the colon crypt [34].

In conclusion, we still do not understand the precise molecular events that lead to the development of a CRC with its typical phenotypic changes, but there is clear evidence now for the presence of different subtypes in CRC. Since most research groups used a different platform and/or statistical method to define their subgroups in CRC, the ultimate goal is to find a consensus about the existence of a subgroup and subsequently define a reproducible subclassifier of the existing subgroup that can be used in daily clinical practice.

**Clinical implications**

On one hand, defining the molecular subgroups in CRC will lead to a better understanding of the disease; on the other hand it should help us guide therapy by providing both prognostic and predictive information.

It is recognized now that MSI-positive CRC is a distinct subgroup of CRC with a favorable stage-adjusted prognosis compared with MSS CRC patients [35]. The role of MSI for response to 5-fluorouracil (5-FU) in the adjuvant setting is conflicting. Ribic et al showed that MSI tumors did not seem to benefit from 5-FU based adjuvant chemotherapy and were possibly even harmed [36]. In 2010, Sargent et al performed a pooled analysis with 457 new CRC patients in combination with the 570 previously published CRC patients, and confirmed the previous results [37]. In contrast, in the PETACC3 study, more than 600 stage II and III patients were evaluated in the control arm with 5-FU alone for benefit in 5-year DFS and revealed a statistically significant difference in 5-year DFS in patients with MSI CRC versus MSS CRC, suggesting that the improved prognosis of MSI tumors was maintained under 5-FU treatment [38]. They also showed that the incidence of MSI differed between stage II and stage III CRC and that the prognostic impact was substantially stronger in stage II compared with stage III. Finally, Simic et al included 1686 stage II and III CRC patients in addition to the previously published 457 patients [37,39] of which 344 had MSI (164 stage II and 180 stage III CRC). They confirmed the better prognosis of MSI patients compared with MSS and reported a benefit of 5-FU treatment for stage III CRC patients with MSI tumors, contrary to what was previously reported [36,37]. Unfortunately, they did not study the effect in stage II tumors. However, they performed an analysis on the impact of sporadic MSI versus germline MSI (i.e., HNPCC) on outcome after 5-FU based chemotherapy and surprisingly this showed no benefit of 5-FU therapy in sporadic MSI stage III CRC with an epigenetic origin, in comparison with MSI CRC that originated from a germline defect in one of the MMR genes. It has to be mentioned though that the authors did not perform molecular genetic analysis of germline DNA, but used other criteria to define germline versus sporadic, therefore this finding still needs to be validated in other series. Another adjuvant study, the NSABP C-08, explored adding 1 year of bevacizumab to oxaliplatin based adjuvant chemotherapy in stage II/III CRC [40]. Although the overall result was negative, the authors recently explored retrospectively the effect in patients with MMR defective (dMMR) tumors versus patients with MMR proficient tumors (pMMR). Surprisingly, the study revealed a statistically significant survival benefit from the addition of bevacizumab (HR 0.52) in dMMR tumors in contrast with no benefit in patients with pMMR tumors [41]. They postulate that a possible explanation might be that dMMR tumor cells, because of their hypermutated status and high immunogenicity, at the micrometastatic level have to evade attack from the immune system in order to progress and VEGF-A is one of the main tumor-derived soluble factors that can create an immune suppressive microenvironment.

Besides MSI, also other prognostic factors have been explored, especially in patients with stage II CRC in which the benefit of adjuvant chemotherapy is limited [42]. Several gene-expression classifiers for predicting CRC relapse have been described [29,43,44] such as for example the ColoPrint, an 18-gene signature [29], but still they are not yet routinely used in daily clinical practice, mainly because of lack of validation sets, limited number of patients, retrospectively collected patient series and lack of assessment in a large patient data set by multivariable analysis.

In the two recently published studies in *Nature Medicine*...
[33,34] using gene expression profiling to classify CRC, the authors also correlated their subgroups with benefit from chemotherapy or targeted agents, such as for example response to irinotecan or resistance to cetuximab. Their findings still have to be validated in larger series, which also accounts for the subgroupes as proposed by Budinska et al [30]. However, what was striking in their analysis was the association with their stromal subtype D and the previously published epithelial-mesenchymal transition signature [45], which showed the poorest survival and might be resistant to chemotherapy.

Finally, anatomically and embryologically, CRC is also divided into proximal colon cancer (right from the splenic flexure), distal colon cancer (left from the splenic flexure) and rectal cancer. The proximal colon originates from the midgut, while the distal colon and rectum arise from the hindgut. Also the nourishing arteries and the innervation differs between left and right colon [46]. Different genetic abnormalities have been found in CRC from different sites. Recently, at the annual American Society of Clinical Oncology (ASCO) 2013 conference, several abstracts have been presented with differences in biology, prognosis and response to treatment in CRC originating in the left versus right side of the colon. For example, BRAF mutations seemed mainly prognostic in left, but not in right-sided tumors (Popovic et al, ASCO 2013). Also benefit from cetuximab in KRAS wild-type patients seemed restricted to left sided tumors (Brule et al, ASCO 2013).

In conclusion, our goal should be to take the molecular background of CRC into account in the future design of clinical trials and to find a consensus in which the different types of CRC can be defined and incorporated in the classification systems as those of the WHO. Also, retrospective analysis of published clinical trials may identify drug sensitivity associated with certain subtypes.

References

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