Genetic Alterations of Colorectal Cancer

Subjects: Others
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Colorectal cancer (CRC) is one of the most common types of cancer in the world, and targeted therapy is frequently used in the clinical management of this cancer. An accurate picture of gene abnormalities observed in this cancer is therefore critical. This entry illustrates some of the recent developments underlying the discovery of the numerous genetic abnormalities observed in colorectal cancer that have helped both to better understand how this tumor develops and to identify therapeutic targets for the development of new therapies.

Keywords: cancer ; oncology ; colorectal cancer ; genomics ; gene mutations ; copy number alterations ; tumor evolution ; targeted therapy

1. Introduction

Colorectal cancer (CRC) is one of the most frequent cancers worldwide, corresponding to the second in males and third in females most frequent tumor. CRC is the second most common cause of cancer death in Europe [1].

Colorectal cancer is a highly heterogeneous disease that comprises different tumor phenotypes, characterized by specific molecular and morphological alterations. CRC is caused by genetic alterations that target tumor suppressor genes, oncogenes, and genes related to DNA repair mechanisms. Depending on the origin of these mutations, CRC can be classified as sporadic (70–75%), hereditary (5%), and familial (20–25%). Three major pathways are involved in CRC origin and progression: (a) chromosomal instability (CIN); (b) microsatellite instability (MSI); (c) CpG island methylation phenotype (CIMP). Each of these three different groups displays peculiar pathological, genetic, and clinical characteristics [2].

CIN is the most common (85% of total CRCs) genetic mechanism occurring in CRC. CIN is characterized by the acquisition of a consistent karyotypic variability, aneuploidy, chromosomal and subchromosomal aberrations, gene amplifications and loss of heterozygosity. Allelic losses at the level of chromosome arms 1p, 5q, 17p, 18p, 18q, 20p, and 22q are highly recurrent. A major pathogenic consequence of this CIN consists in the loss of heterozygosity at tumor suppressor gene loci. Furthermore, CIN tumors are associated with the accumulation of mutations at the level of several oncogenes, including KRAS and BRAF and of tumor suppressor genes such as APC and TP53. The meta-analysis of the outcome of more than 10,000 CRC patients clearly indicated that CIN is associated with a worse prognosis [3].

2. Molecular abnormalities of CRC

The Cancer Genome Atlas provided in 2002 the first genome-scale analysis of a large set (276) of CRC samples, performing a comprehensive study involving exome sequencing, DNA copy number, promoter methylation, messenger RNA and micro RNA expression evaluation [4]. This analysis showed that CRCs can be classified according to their mutation pattern: (i) 16% of CRCs were found to be hypermutated (75% displayed high MSI, usually associated with hypermethylation and silencing of the *MLH1* gene, whereas the remaining 25% exhibited mismatch-repair gene and polymerase ε (POLE) gene mutations); (ii) the non-hypermutated CRCs that formed the most consistent group of tumors showed the recurrent mutations of *APC*, *TP53*, *KRAS*, *PIK3CA*, *FBXW7*, *SMAD4*, *TCF7L2,* and *NRAS* genes; (iii) in hypermutated CRCs, the most frequently mutated genes were *ACVR2A* (63%), *APC* (51%), *TGFBR2* (51%), *BRAF* (49%), *MSH3* (46%), *MSH6* (40%), *MYO18* (31%), *TCF7L2* (31%), and *CASP8* (29%); (iv) *APC* (81% vs. 51%) and *TP53* (60% vs. 20%) were significantly more mutated in the non-hypermutated cancers compared to hypermutated cancers. Integrated analysis of the genetic profiling showed that some pathways are recurrently altered in CRCs: (i) WNT pathway is altered in 93% of all tumors (in 80% of cases due to biallelic inactivation of *APC* or activating mutations of *CTNNB1*); (ii) PI3K signaling pathway is altered in 50% of non-hypermutated and 53% of hypermutated CRCs; (iii) RTK-RAS signaling pathway is more frequently altered in hypermutated (80%) than in non-hypermutated (59%); (iv) finally, TGF-β signaling pathway was much more frequently altered in hypermutated (87%) than in non-hypermutated (27%) CRCs [4].

The analysis of gene expression profiles obtained through the study of thousands cases of colorectal cancers supported a classification of colon cancer, based on four major consensus molecular subtypes (CMS), CMS1 to CMS4 (Table 1) [5]. CMS1 group (MSI immune subtype, including 14% of all CRCs) is characterized at genetic level by hypermutation, hypermethylation, enrichment for *BRAF*^{V600E} mutations (observed in 40% of these tumors) and by pronounced infiltration of the tumor microevironment by immune cells, particularly represented by T lymphocytes (both Cytotoxic $CD8^+$ and $CD4^+$ T helper) and natural killer lymphocytes; frequent in these tumors are mutations at the level of *APC* (35%), *TP53* (30%) and *KRAS* (25%) genes. Frequent in these tumors are mutations in *MSH6*, *RNF43*, *ATM*, *TGFBR2*, *BRAF,* and *PTEN* genes. Predominantly, these tumors originate from precursor lesions with a serrated histology, with preferential location at the level of proximal regions of the colon; their prognostic outcome is intermediate but poor after relapse. The CMS2 subtype corresponds to the canonical subtype (37% of CRCs) and is characterized by CIN-high, microsatellite stability (MSS) and low levels of gene hypermethylation; a mutational profile typically observed in CIN-high CRCs, including recurrent *APC* (75%), *TP53* (70%), and *KRAS* (30%) mutations, whereas *BRAF* mutations were absent; pronounced upregulation of WNT and MYC downstream targets, elevated expression of EGFR, HER2, IGF2, IRS2, HNF4A, and cyclin; complex tubular histological structure, predominantly located in the distal region of the colon. The CMS3 subtype corresponds to the metabolic subtype (10% of CRCs) that is characterized by activation of glutaminolysis and lipidogenesis and by the presence of a distinctive genomic and epigenomic profile compared with other CIN tumors, for the presence of a mixed CIMP-H (20% of cases), MSI-H (15% of cases), hypermutation (30% of cases), and CIN-H (54% of cases); at mutational level, frequent *KRAS* and *APC* mutations but less frequent *TP53* and *BRAF* mutations are observed; these tumors predominantly display papillary morphology and are located at the level of both proximal and distal regions of colon. CMS4 corresponds to the mesenchymal subtype (25% of all cases) and is characterized by the presence of tumors exhibiting activation of the pathways related to epithelial-mesenchymal transition (EMT) and stemness (TGF-β signaling and integrins) and overexpression of genes involved in extracellular matrix remodeling, complement-associated inflammation, stromal invasion and angiogenesis; marked stromal cell infiltration at the level of peritumoral microenvironment is a typical histological feature of these tumors; these tumors are frequently CIN-H but rarely hypermutated, CIMP-H and MSI-H; at mutational level, frequent are the mutations of *APC*, *TP53* and *KRAS*, associated with rare *BRAF* mutations; at histological level, these tumors are characterized by a desmoplastic reaction with high stroma; these tumors are associated with a poor outcome compared with the other CMS subtypes [5].

Finally, there is a residual unclassified group representing 10–15% of all tumors with mixed features, that seemingly represents a transitional phenotype or reflects an intra-tumoral heterogeneity [5].

3. Effect of Therapy on Mutational Landscape of Metastatic CRC

The targeted therapy of metastatic CRC patients implies the exploration of the targeted biomarker and its presence in both the primary and the metastatic tumors. The introduction of EGFR inhibitors for treatment of metastatic CRC patients allowed the unique opportunity to obtain, through the analysis of numerous clinical studies, data on the concordance of the mutational status for KRAS, NRAS, BRAF and PIK3CA between primary tumors and metastases in more than 3500 patients [6]. This meta-analysis involving 61 clinical studies and data on 3565 metastatic CRCs, showed: (i) a median biomarker concordance for KRAS (93.7%), NRAS (100%), BRAF (99.4%), and PIK3CA (93%); (ii) a pooled discordance of 8% for KRAS, 8% for BRAF, and 7% for PIK3CA [6]. These observations further support the maintenance of the main driver gene alterations in CRCs undergoing metastatic spreading [6]. The detection of KRAS mutations in metastatic CRC is important because implies a negative prognosis and a poor response to standard chemotherapy [7].

An important example of the therapy-driven effects on the genomic alterations of metastatic CRC derives from the analysis of patients developing resistance to therapies based on EGFR inhibitors. EGFR inhibitors are effective in a subset of KRAS wild-type metastatic CRCs; however, after an initial response, the development of secondary resistance mechanisms cause disease relapse, thus limiting the clinical benefit of this treatment: The analyses of metastases of patients who developed resistance to EGFR inhibitors showed more rarely the emergence of KRAS amplification and more frequently the acquisition of secondary KRAS mutations; in these patients, KRAS mutant alleles were detectable in the blood circulating tumor DNA 10 months before the radiographic documentation of disease progression [8]. These observations suggest that EGFR-targeted therapy exerts a selective effect on CRCs either inducing the expansion of pre-existing KRAS-mutant subclones or favoring the development of new KRAS alterations [8]. Another mechanism of secondary resistance to EGFR blockade is represented by novel alterations of ectodomain of EGFR [9]. The study of individual patients has shown that different metastatic biopsies from the same patient with CRC display genetically distinct mechanisms of resistance to EGFR blockade: thus, in some patients, it was documented that distinct resistance mechanisms emerge in different metastases in the same patient and can drive lesion-specific responses to different targeted therapies [9].

Genetic mechanisms of primary resistance to EGFR inhibitors among KRAS wild-type CRC patients are represented by NRAS mutations, ^{V600E}BRAF mutations, MET amplification, ERBB2 amplification, PIK3CA mutations at the level of exon 20, mutations in FGFR1, PDGFRA, and MAP2K1, and homozygous deletions of PTEN [10].

Using xenografts derived from hepatic metastases of CRC patients, amplification of ERBB2 was identified as a potential therapeutic target in cetuximab-resistant CRCs [11]. These preclinical observations supported a clinical study (HERACLES) evaluating trastuzumab and lapatinib in metastatic CRC patients with amplified ERBB2 refractory to standard cares: in 33 patients, 24.2% objective responses were observed with durable clinical benefit lasting >24 months in responding patients [11]. Although ERBB2 blockade was effective, most of responding patients relapse [12]. A recent study explored the mechanisms of tumor evolution responsible for relapse to HER2 blockade. In fact, the analysis of circulating tumor DNA allowed to define organ and metastases-private evolutionary patterns and high-levels in intra-patient molecular heterogeneity, defining lesion-specific evolutionary trees and potential pharmacologic vulnerabilities [13].

4. Models of CRC Progression and Evolution

The study of tumor heterogeneity is a fundamental tool to analyze and to define the molecular and cellular mechanisms responsible for the development of CRC and have provided a consistent contribution to the development of current theories to explain CRC development.

Two different models have been proposed in the time to explain the origin and development of CRC metastasis: one suggesting a common origin for both the primary tumor and metastases and the other hypothesizing a completely independent genesis of metastases and of the primary tumor. The sequencing data of matched primary tumors and metastases have strongly supported the existence of a common ancestor of both the primary tumor and of the corresponding metastases.

The development of CRC from a common ancestor implies two different models to explain metastasis evolution: the parallel progression model suggests that the dissemination of metastasizing tumor cells occurs during early stages of primary tumor and the primary tumor and metastases evolve separately thereafter. The linear progression model implies the occurrence of metastases as a sequential event occurring during primary tumor development.

5. Conclusions

About half of CRCs develop metastases and metastatic spreading is the main cause of CRC-related death. The dynamics and the molecular processes remain largely unknown. Several recent studies have shown that systemic spread can occur early in CRC development. Recent studies have reported a detailed analysis of the genomic landscape of metastatic CRC patients underlying the molecular heterogeneity of these patients and the possibility to identify some therapeutic targets in these patients. The study of molecular evolution of CRCs suggest that these tumors may evolve either through a process of subclonal selection or neutral evolution.

A better understanding of the cellular and molecular processes governing CRC metastasis spreading will be necessary to improve the outcome of metastatic CRC patients.

Although the survival rate of patients with metastatic CRC patients improved in the last years, the response to current treatments and prognosis of patients bearing KRAS, NRAS, and BRAF mutations remain still poor. Therefore, there is an absolute need to identify these patients and to discover new improvements for therapeutic vulnerabilities and to formulate rational prospective personalized therapies aiming to improve their survival chances.

References

1. Dekker, E.; Tanis, P.J.; Valengels, J.; Kass, P.M.; Wallace, M.B. Colorectal cancer. Lancet 2019, 394, 1467–1480.
2. Mullert, M.F.; Ibrahim, A.; Arends, M.J. Molecular pathological classification of colorectal cancer. Wirchows Arch. 2016, 469, 125–134.
3. Walther, A.; Houlston, R.; Toulinson, I. Association between chromosomal instability and prognosis in colorectal cancer: A meta-analysis. Gut 2008, 57, 941–950.
4. The Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nat ure 2012, 487, 330–337.

5. Guinney, J.; Dienstmann, R.; Wang, X.; De Reynies, A.; Schilker, A.; Soneson, C.; Marisa, L.; Roepman, P.; Nyamundanda, G.; Angelino, P.; et al. The consensus molecular subtypes of colorectal cancer. Nat. Med. 2015, 21, 1350–1356.
6. Bhullar, D.S.; Barriuso, J.; Mullamitha, S.; Saunders, M.P.; O'Dwyer, S.T.; Aziz, O. Biomarker concordance between primary colorectal cancer and its metastases. EBioMedicine 2019, 40, 363–374.
7. Garcia-Carbonero, N.; Martinez-Useros, J.; Li, W.; Orta, A.; Perez, N.; Carames, C.; Hernandez, T.; Moreno, I.; Serrano, G.; Garcia-Foncillas, J. KRAS and BRAF mutations as prognostic and predictive biomarkers for standard chemotherapy response in metastatic colorectal cancer: A single institutional study. Cells 2020, 9, 219.
8. Misala, S.; Yaeger, R.; Hober, S.; Scala, E.; Janckraman, M.; Liska, D.; Valtorta, E.; Schiavo, R.; Buscarino, M.; Siravegna, G.; et al. Emergence of KRAS mutations and acquired resistance to anti EGFR therapy in colorectal cancer. Nature 2012, 486, 532–536.
9. Bertotti, A.; Papp, E.; Jones, S.; Adleff, V.; Anagnostou, V.; Lupo, B.; Sausen, M.; Phallen, J.; Hrubau, C.A.; Tokheim, C.; et al. The genomic landscape of response to EGFR blockade in colorectal cancer. Nature 2015, 526, 263–267.
10. Russo, M.; Siravegna, G.; Blazkowsky, L.S.; Corti, G.; Crisafulli, G.; Ahronian, L.G.; Mussolin, B.; Kwak, E.L.; Buscorino, M.; Lazzari, L.; et al. Tumor heterogeneity and lesion-specific response to targeted therapy in colorectal cancer. Cancer Discov. 2016, 6, 147–153.
11. Bertotti, A.; Milgiardi, G.; Galimi, F.; Sassi, F.; Torti, D.; Isella, C.; Corà, D.; Di Nicolantonio, F.; Buscarino, M.; Petti, F.; et al. A molecularly annotated platform of patient-derived xenografts ("xenopatients") identified HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov. 2011, 1, 508–523.
12. Sartore-Bianchi, A.; Trusolino, L.; Martino, C.; Bencardino, K.; Lonardi, S.; Bergamo, F.; Zagonel, V.; Leone, F.; Depetris, I.; Martinelli, E. Dual-targeted therapy with trastuzumab and lapatinib in tretament-refractory, KRas codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): A proof-of-concept, multicentre, open-label phase 2 trial. Lancet Oncol. 2016, 17, 738–746.
13. Siravegna, G.; Lazzari, L.; Crisafulli, G.; Sartore-Bianchi, A.; Mussolin, B.; Cossingena, A.; Martiono, C.; Lanman, R.B.; Nagy, R.J.; Fairalough, S.; et al. Radiologic and genomic evolution of individual metastases during HER2 blockade in colorectal cancer. Cancer 2018, 34, 148–162.
