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New insights into the molecular pathogenesis of colorectal cancer

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Abstract

Although there have been tremendous advances in the management of colorectal cancer (CRC), there is still a need for improved therapeutic approaches. On a molecular genetic level, CRC is one of the best-understood solid malignancies, and these insights can serve as a foundation for the design of novel targeted therapies. We present new genetic and epigenetic pathways that highlight the heterogeneous mechanisms in CRC pathogenesis, including the roles of the MYH DNA repair gene and of aberrant DNA hypermethylation and imprinting. We then describe some of the successful targeted therapies that inhibit COX2, EGFR, and VEGF as well as potential new targets that have been revealed by studies of molecular genetics.

Introduction

In 2006, it is estimated there will be nearly 55,000 colorectal cancer (CRC)-related deaths in the United States, making this disease the second-leading cause of cancer-related mortality [1]. Whereas the 5-year survival for stage I CRC is greater than 90%, it falls to less than 10% for stage IV disease [2]. Despite recent advances in the management of CRC, there remains a compelling need for new therapies. The underlying molecular pathogenesis of CRC is one of the best understood among solid tumors, and these insights can and have served as the basis for the development of targeted therapies. We briefly summarize some of the key genetic mechanisms in CRC pathogenesis, highlight several new insights, and describe the current status of targeted therapies.

Overview of CRC genetics

Genetic instability underlies the pathogenesis of all CRCs. The mechanistic basis is traditionally divided into two broad categories: (1) CHROMOSOMAL INSTABILITY (CIN) (see Glossary), which is observed in about 85% of all cases and (2) MICROSATELLITE INSTABILITY (see Glossary) (MSI), which comprises the remaining 15%.

A model of colorectal carcinogenesis based on the first category, CIN, has been proposed in which a specific mutation is required at each stage of progression along the adenoma–carcinoma sequence (Fig. 1). Mutations in the Adenomatous Polyposis Coli (*APC*) gene (GenBank accession no. 324) are typically the critical initiating event in cases of FAMILIAL ADENOMATOUS POLYPOSIS (FAP)-associated as well as most sporadic cancers (see Glossary), resulting in activation of the Wnt signaling pathway. This is followed by mutations in the *KRAS* oncogene (GenBank accession no. 3845) and loss of heterozygosity (LOH) of chromosome 18q, where tumor suppressor genes including deleted in colon cancer (*DCC*) (GenBank accession no. 1630), *SMAD4* (GenBank accession no. 4089), *SMAD6* (GenBank accession no.

4091), and the human homolog of *Cables* (GenBank accession no. 63955) lie. *TP53* (GenBank accession no. 7157) mutations are a late event, occurring during the transition from the advanced adenoma to invasive carcinoma [3].

Studies of patients with the HEREDITARY NON-POLYPOSIS COLON CANCER (HNPCC) syndrome (see Glossary) have revealed a second genetic pathway, the so-called MSI pathway. Mutations in one of the DNA mismatch repair enzymes *MLH1* (GenBank accession no. 4292), *MSH2* (GenBank accession no. 4436), *MSH6* (GenBank accession no. 2956), or *PMS2* (GenBank accession no. 5395) result in insertion or deletion errors at DNA polynucleotide repeats, also known as microsatellites. The resulting expansions and contractions of these repeats define the MSI phenotype. All patients with HNPCC and 15% of sporadic cases exhibit MSI. For further details, please see one of the many excellent reviews [4].

The MYH (*MUTYH*) gene

The *E. coli* *mutY* (GenBank accession no. 947447) gene plays a key role in DNA base excision repair. When altered, there is a genome-wide increase in G:C → T:A transversion mutations. In a family with multiple colorectal adenomas and carcinomas without evidence for an inherited *APC* mutation, 11 tumors from three affected siblings revealed somatic inactivating mutations of *APC* due to G:C → T:A transversions [5]. Further analysis revealed that both alleles of the human homolog of *mutY* (MYH or *MUTYH*) gene (GenBank accession no. 4595) were mutated in the germline in all cases. In larger studies of patients with multiple colorectal adenomas and carcinomas, homozygous or compound heterozygous mutations in *MUTYH* have been identified in as many as 23–36% of patients [6]. The *APC* gene frequently acquires somatic mutations when MYH is dysfunctional, and this may explain some of the overlapping clinical features with the FAP syndrome [7].

The MYH-associated polyposis syndrome is the first cancer susceptibility disorder to exhibit an autosomal recessive inheritance pattern. Because of its variable clinical phenotype, there can be overlap with other hereditary CRC syndromes. In patients with clinical features of FAP or attenuated FAP, as many as 29% have been ultimately shown to harbor biallelic mutations in *MUTYH* [8]. Similarly, in 137 patients from HNPCC-like families without detectable DNA mismatch repair gene mutations, the ODDS RATIO for a disease-causing *MUTYH* mutation was 3.23 (see Glossary), indicating that many HNPCC-like families are likely to have a disease-causing *MUTYH* mutation [9].

Although inheritance of mutations in both copies of *MUTYH* clearly increases the risk of polyposis and cancer, recent evidence suggests that heterozygotes may display as high as a three-fold increased risk for CRC [10]. Heterozygosity of *MUTYH* may potentially explain a proportion of familial cancers in which the predisposing mutation is unknown. With an estimated gene frequency in the population as high as 1%, the implications for cancer risk due to *MUTYH* mutations are not insignificant. The DNA base excision repair system has not been previously implicated in CRC pathogenesis and therefore represents a new potential target for therapy.

DNA hypermethylation

In addition to genetic mutations, EPIGENETIC CHANGES can underlie tumor pathogenesis (see Glossary). The most common epigenetic alteration in colon cancer is aberrant DNA methylation, in which a methyl group is added to the cytosine base in the dinucleotide sequence CpG. There are regions of increased density of CpG dinucleotides in the 5' promoter region of many genes, and heavy methylation of these CpG islands results in transcriptional inactivation, as exemplified by the silenced X chromosome in females or the silenced allele of IMPRINTED GENES (see

Glossary) (Fig. 2). Binding of methylation-specific proteins can modify histones and control the transition of chromatin from the active to the inactive state [11].

Cancer cells often demonstrate a loss of global methylation, but there are increases in methylation at CpG islands in specific promoter regions [12]. Certain genes are consistently methylated at 3–5-fold higher levels in malignancies. This is most pronounced in CRCs and has been termed the CPG ISLAND METHYLATOR PHENOTYPE (CIMP) (see Glossary). Although there is an age-dependent increase in DNA methylation in normal colonic mucosa, an even higher rate of methylation is observed in as many as 50% of patients with CRC [13]. Of note, sporadic CRCs with MSI typically exhibit promoter hypermethylation of the DNA mismatch repair gene *MLH1*. De-methylation of *MLH1* results in the loss of MSI [14]. Other genes that are frequently methylated include *CDKN2A* (GenBank accession no. 1029), thrombospondin I (*THBS1*) (GenBank accession no. 7057), and O6-methylguanine-DNA methyltransferase (*MGMT*) (GenBank accession no. 4255)[15].

The existence of a distinct ‘CIMP phenotype’ has been controversial, as it has been postulated that many of the clinical characteristics associated with CIMP overlapped with the MSI phenotype [16]. A recent rigorous analysis of 195 CpG islands in 295 tumor samples carefully defined a new set of five markers, and methylation of these markers was highly associated with mutations in *BRAF* (GenBank accession no. 673) instead of *KRAS*, and accounted for almost all sporadic tumors with MSI [17]. Thus, there does appear to be considerable overlap between these two pathways, but it is yet not clear whether there may be a distinct subgroup of CIMP tumors without MSI. Further studies are necessary to identify the mechanisms and specific DNA methyltransferases that regulate this tumor-specific promoter hypermethylation, as inhibition of DNA methylation represents a potential targeted strategy that would be relevant to a significant number of CRCs.

Serrated polyps

Traditionally, colorectal polyps have been divided into two major histological categories: (1) adenomatous and (2) hyperplastic. It has been considered axiomatic that adenomatous polyps were precursors of CRC through the classic adenoma–carcinoma sequence, whereas hyperplastic polyps did not have malignant potential [3]. However, recent evidence suggests that a subset of these hyperplastic polyps might participate in an alternative carcinogenic pathway that result in CRCs with MSI [18].

Serrated polyps have histological characteristics of both hyperplastic and adenomatous polyps. Due to an increase in the cellular proliferation zone and inhibition of apoptosis, these lesions display a ‘saw-toothed’ or serrated crypt architecture, reminiscent of classical hyperplastic polyps. However, they are also lined with dysplastic epithelial cells, characteristic of classical adenomatous polyps [19]. The malignant potential of these lesions was first appreciated in an initial study of 110 serrated polyps, as 37% contained foci of significant dysplasia and 11% contained intramucosal carcinoma [20]. It has since been shown that patients with serrated polyps have a similar risk for the subsequent development of CRC as those with adenomatous polyps [21].

Speculation of an alternative pathway of colorectal carcinogenesis was fueled by the fact that significantly lower rates of mutations in the *APC*, β -catenin (*CTNNB1*) (GenBank accession no. 1499), *KRAS* and *TP53* genes were noted in serrated polyps when compared to conventional adenomas [22]. In a departure from the traditional adenoma–carcinoma model, there are high rates of *BRAF* instead of *KRAS* mutations in serrated polyps [23]. Furthermore, a strong association with hypermethylation and MSI has been demonstrated in these polyps [24]. The frequent identification of *BRAF* mutations in the context of the CIMP phenotype in serrated

polyps as well as in sporadic CRCs with MSI implies that these serrated polyps may indeed be precursor lesions for this subset of CRC (Fig. 1)[25].

Loss of imprinting of IGF-II

Insulin-like growth factor II (*IGF2*) (GenBank accession no. 3481) is an imprinted gene in which the maternal allele is silenced through epigenetic mechanisms (see Glossary). It is an important autocrine growth factor for many tumors as a result of its mitogenic and anti-apoptotic functions [26]. These effects of *IGF2* are mediated through an interaction with the insulin-like growth factor type I receptor (*IGF1R*) (GenBank accession no. 3480), resulting in activation of the phosphatidylinositol 3-kinase and protein kinase B/Akt pathways. Akt can regulate a wide variety of processes including: (1) inactivation of glycogen synthase kinase 3 β (*GSK3- β*) (GenBank accession no. 2932), blocking the degradation of cyclin D1 (*CCND1*) (GenBank accession no. 595); (2) increased translocation of mouse double minute 2 (*MDM2*) (GenBank accession no. 4193) to the nucleus, resulting in increased degradation of *TP53*; (3) inhibition of the positive regulation of apoptosis by bcl-associated death promoter (*BAD*) (GenBank accession no. 572), and (4) activation of the target of rapamycin (mTOR) (GenBank accession no. 47396) pathway [26].

Loss of imprinting (LOI) of the maternal copy of the *IGF2* gene was first observed in Wilms tumor and subsequently identified in a variety of cancers, including as many as 44% of CRCs [27,28]. LOI of the *IGF2* gene results in increased levels of *IGF2* expression (Fig. 3). Interestingly, such LOI has been identified in the germline, and the odds ratio of LOI of *IGF2* in peripheral lymphocytes was 3.5 for those with a history of adenoma and between 5.15 and 21.7 in those with a history of CRC [29]. In addition, LOI of *IGF2* is common (27%) in the general population and those with LOI of *IGF2* in normal colonic mucosa have a five-fold increased risk of adenoma formation [30]. Interestingly, poorly differentiated or mucinous carcinomas and proximal lesions were strongly associated with LOI, but there was no statistically significant difference in LOI of *IGF2* between tumors with or without MSI [31].

A mouse model was generated to investigate the mechanism by which LOI of *IGF2* may promote intestinal tumorigenesis [32]. Imprinting of *IGF2* is regulated by a differentially methylated region upstream of the nearby untranslated *H19* (GenBank accession no. 283120) gene. Female *H19*^{+/-} animals were mated with male *Apc*^{Min+/-} mice. Offspring that inherited the mutant *H19* gene demonstrated LOI of *IGF2* and developed twice as many intestinal tumors. In these mice, there was an increase in crypt length and staining with markers for undifferentiated progenitor cells. It was hypothesized that activation of *IGF2* increases the population of undifferentiated cells, thereby increasing the pool of cells susceptible to acquiring carcinogenic mutations. The regulation of imprinting of *IGF2* in humans remains poorly defined, but is likely to depend upon specific DNA methyltransferases as well as DNA demethylases that have yet to be identified. Both will represent therapeutic targets in the future. Another strategy that currently targets the IGF2 signaling pathway is blockade of the IGF receptor, and Phase I trials of novel agents are underway (Table 1).

Current targeted therapies

Efforts to target some of the key genes that are mutated in CRC have not yet enjoyed great success clinically. For example, targeting K-ras with farnesyltransferase inhibitors was disappointing in initial clinical trials. However, several agents that instead target downstream Ras effectors such as Raf, MEK or Akt are now being studied in Phase I trials (Table 1). Small molecule inhibitors such as ICG-001 (Institute for Chemical Genomics, <http://www.ichemgen.org/>) that can block the β -catenin/TCF transcriptional complex that activates Wnt pathway genes have recently been identified and are being tested pre-clinically [33]. Interestingly, the most successful agents to date do not target the genes that are specifically

mutated in CRC. Rather, they inhibit genes that are expressed at higher levels in CRC, often as a consequence of enhanced signaling through pathways such as K-ras and Wnt. For example, overexpression of cyclo-oxygenase 2 (*PTGS2*) (GenBank accession no. 5743) is seen in early adenomas and promotes cell division, neovascularization, and metastasis, while also inhibiting apoptosis [34]. Sulindac (Merck, <http://www.merck.com/>) and celecoxib (Pfizer, <http://www.pfizer.com/>) can inhibit COX2 and are now FDA-approved for polyp prevention in selected patients with FAP. However, the recent association of COX2-selective inhibitors with an increase in cardiovascular toxicities brings the future of these drugs as chemopreventive agents into question [35]. The epidermal growth factor receptor (*EGFR*) (GenBank accession no. 1956) is upregulated in up to 80% of CRCs and controls cell differentiation, proliferation, apoptosis, and angiogenesis [36]. Cetuximab (Merck, <http://www.merck.com/>), an anti-EGFR monoclonal antibody, alone or in combination with irinotecan (Pfizer, <http://www.pfizer.com/>) demonstrated significantly increased activity against irinotecan-resistant tumors [37]. A recent Phase III trial of panitumumab (Amgen, <http://www.amgen.com/>), a humanized anti-epidermal growth factor receptor monoclonal antibody, as a single agent for previously treated metastatic colon cancer also showed a 46% risk reduction in tumor progression and a partial response rate of 8%. Further studies are underway to evaluate small molecule EGFR-tyrosine kinase inhibitors such as ZD1839/gefitinib (Astra Zeneca, <http://www.astrazeneca.com/>) and erlotinib (Genentech, <http://www.genentech.com/>). Finally, overexpression of vascular endothelial growth factor (VEGF) (GenBank accession no. 7422) is critical for the induction of angiogenesis in CRC [38]. Bevacizumab (Genentech, <http://www.genentech.com/>), an α -VEGF humanized monoclonal antibody, in conjunction with chemotherapy can significantly improve survival for patients with metastatic CRC [39]. Small molecule VEGF-receptor tyrosine kinase inhibitors such as PTK 787/ZK 222584 (Schering, <http://www.schering.de/>) are currently under investigation as well. For further details on these and other inhibitors, see Table 1.

Conclusions

Our understanding of CRC genetics continues to expand from the initial framework laid out by Vogelstein and colleagues [3]. The identification of new genes such as MYH as well as the recognition of alternative pathways such as the serrated polyp-carcinoma sequence and the epigenetic alterations in DNA methylation and imprinting highlights the heterogeneous mechanisms of CRC pathogenesis. These findings provide potential new targets for drug development but also imply that these therapies may be successful only in certain CRC subsets. Currently, targeted therapies that are used clinically inhibit COX2, EGFR, and VEGF. Although approaches that target mutated forms of *KRAS* have been largely unsuccessful, some of the downstream effectors of K-ras, such as Raf, MEK, PI3-K, or Akt, can serve as potential targets and are currently being tested [40]. Deficient DNA repair underlies a significant proportion of CRCs, but strategies to restore defective DNA repair activity are particularly challenging and will likely require the delivery of gene replacement therapies. However, the increasing recognition of the key role of aberrant DNA methylation and de-methylation in colon cancer implies that the specific DNA methyltransferases and demethylases that control these processes may be very promising targets in the future.

Glossary

Chromosomal
instability

instability at the chromosomal level in the form of extra or missing chromosomes or duplications or deletions in regions of particular chromosomes.

CpG island mutator phenotype	cancer cells often can exhibit a global loss of methylation, but increased methylation at specific CpG islands in promoter regions.
Epigenetic change	a set of inheritable, but reversible changes in gene function that occurs without a change in DNA sequence.
Familial adenomatous polyposis	a syndrome in which an inherited mutation in the APC gene that results in hundreds to thousands of colonic polyps by the third decade of life.
Hereditary non-polyposis colon cancer	a syndrome with inherited mutation in DNA mismatch repair enzymes that results in an increased incidence in colonic and gynecological tumors.
Imprinted genes	chromosomes, segments of chromosomes, or some genes, can be stamped with a 'memory' of the parental chromosome. This can often be in the form of DNA methylation.
Microsatellite instability	increases or decreases in the lengths of nucleotide repeats (microsatellites) secondary to defective DNA mismatch repair.
Odds ratio	the odds ratio is a method to compare the probability of a certain event in two different cohorts.

References

1. Jemal A, et al. Cancer statistics. *CA Cancer J. Clin* 2006;56:106–130. [PubMed: 16514137]
2. O'Connell JB, et al. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. *J. Natl. Cancer Inst* 2004;96:1420–1425. [PubMed: 15467030]
3. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996;87:159–170. [PubMed: 8861899]
4. Jeter JM, et al. Genetics of colorectal cancer. *Oncology (Williston Park)* 2006;20:269–276. discussion 285–266, 288–269. [PubMed: 16629258]
5. Al-Tassan N, et al. Inherited variants of MYH associated with somatic G:C → T:A mutations in colorectal tumors. *Nat. Genet* 2002;30:227–232. [PubMed: 11818965]
6. Venesio T, et al. High frequency of MYH gene mutations in a subset of patients with familial adenomatous polyposis. *Gastroenterology* 2004;126:1681–1685. [PubMed: 15188161]
7. Lipton L, et al. Carcinogenesis in MYH-associated polyposis follows a distinct genetic pathway. *Cancer Res* 2003;63:7595–7599. [PubMed: 14633673]
8. Sieber OM, et al. Multiple colorectal adenomas, classic adenomatous polyposis, and germ-line mutations in MYH. *N. Engl. J. Med* 2003;348:791–799. [PubMed: 12606733]
9. Peterlongo P, et al. Increased frequency of disease-causing MYH mutations in colon cancer families. *Carcinogenesis*. 2006 10.1093/carcin/bgl1093 Epub ahead of print, June 14.
10. Jenkins MA, et al. Risk of colorectal cancer in monoallelic and biallelic carriers of MYH mutations: a population-based case-family study. *Cancer Epidemiol. Biomarkers Prev* 2006;15:312–314. [PubMed: 16492921]
11. Bjornsson HT, et al. An integrated epigenetic and genetic approach to common human disease. *Trends Genet* 2004;20:350–358. [PubMed: 15262407]
12. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N. Engl. J. Med* 2003;349:2042–2054. [PubMed: 14627790]

13. Toyota M, et al. CpG island methylator phenotype in colorectal cancer. *Proc. Natl. Acad. Sci. U S A* 1999;96:8681–8686. [PubMed: 10411935]
14. Herman JG, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc. Natl. Acad. Sci. U S A* 1998;95:6870–6875. [PubMed: 9618505]
15. Shen L, et al. MGMT promoter methylation and field defect in sporadic colorectal cancer. *J. Natl. Cancer Inst* 2005;97:1330–1338. [PubMed: 16174854]
16. Yamashita K, et al. Genetics supersedes epigenetics in colon cancer phenotype. *Cancer Cell* 2003;4:121–131. [PubMed: 12957287]
17. Weisenberger DJ, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat. Genet* 2006;38:787–793. [PubMed: 16804544]
18. Jass JR. Serrated adenoma of the colorectum and the DNA-methylator phenotype. *Nat. Clin. Pract. Oncol* 2005;2:398–405. [PubMed: 16130936]
19. Cunningham KS, Riddell RH. Serrated mucosal lesions of the colorectum. *Curr. Opin. Gastroenterol* 2006;22:48–53. [PubMed: 16319676]
20. Longacre TA, Fenoglio-Preiser CM. Mixed hyperplastic denomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. *Am. J. Surg. Pathol* 1990;14:524–537. [PubMed: 2186644]
21. Lazarus R, et al. The risk of metachronous neoplasia in patients with serrated adenoma. *Am. J. Clin. Pathol* 2005;123:349–359. [PubMed: 15716230]
22. Sawyer EJ, et al. Molecular characteristics of serrated adenomas of the colorectum. *Gut* 2002;51:200–206. [PubMed: 12117880]
23. Yang S, et al. BRAF and KRAS Mutations in hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology and CpG island methylation status. *Am. J. Surg. Pathol* 2004;28:1452–1459. [PubMed: 15489648]
24. Konishi K, et al. Molecular differences between sporadic serrated and conventional colorectal adenomas. *Clin. Cancer Res* 2004;10:3082–3090. [PubMed: 15131047]
25. Kambara T, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. *Gut* 2004;53:1137–1144. [PubMed: 15247181]
26. Kaneda A, Feinberg AP. Loss of imprinting of IGF2: a common epigenetic modifier of intestinal tumor risk. *Cancer Res* 2005;65:11236–11240. [PubMed: 16357124]
27. Rainier S, et al. Relaxation of imprinted genes in human cancer. *Nature* 1993;362:747–749. [PubMed: 8385745]
28. Cui H, et al. Loss of imprinting in normal tissue of colorectal cancer patients with microsatellite instability. *Nat. Med* 1998;4:1276–1280. [PubMed: 9809551]
29. Cui H, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. *Science* 2003;299:1753–1755. [PubMed: 12637750]
30. Woodson K, et al. Loss of insulin-like growth factor-II imprinting and the presence of screen-detected colorectal adenomas in women. *J. Natl. Cancer Inst* 2004;96:407–410. [PubMed: 14996863]
31. Sasaki J, et al. Clinicopathological characteristics of colorectal cancers with loss of imprinting of insulin-like growth factor 2. *Int. J. Cancer* 2006;119:80–83. [PubMed: 16432831]
32. Sakatani T, et al. Loss of imprinting of Igf2 alters intestinal maturation and tumorigenesis in mice. *Science* 2005;307:1976–1978. [PubMed: 15731405]
33. Eguchi M, et al. ICG-001, a novel small molecule regulator of TCF/beta-catenin transcription. *Med. Chem* 2005;1:467–472. [PubMed: 16787331]
34. Shiff SJ, Rigas B. The role of cyclooxygenase inhibition in the antineoplastic effects of nonsteroidal antiinflammatory drugs (NSAIDs). *J. Exp. Med* 1999;190:445–450. [PubMed: 10449515]
35. Grosch S, et al. Cyclooxygenase-2 (COX-2)-independent anticarcinogenic effects of selective COX-2 inhibitors. *J. Natl. Cancer Inst* 2006;98:736–747. [PubMed: 16757698]
36. Ciardiello F, Tortora G. Epidermal growth factor receptor (EGFR) as a target in cancer therapy: understanding the role of receptor expression and other molecular determinants that could influence the response to anti-EGFR drugs. *Eur. J. Cancer* 2003;39:1348–1354. [PubMed: 12826036]
37. Cunningham D, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N. Engl. J. Med* 2004;351:337–345. [PubMed: 15269313]

38. Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J. Clin. Oncol* 2005;23:1011–1027. [PubMed: 15585754]
39. Hurwitz H, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N. Engl. J. Med* 2004;350:2335–2342. [PubMed: 15175435]
40. Rychahou PG, et al. Targeted molecular therapy of the PI3K pathway: therapeutic significance of PI3K subunit targeting in colorectal carcinoma. *Ann. Surg* 2006;243:833–842. [PubMed: 16772787]
41. Crul M, et al. Phase I clinical and pharmacologic study of chronic oral administration of the farnesyl protein transferase inhibitor R115777 in advanced cancer. *J. Clin. Oncol* 2002;20:2726–2735. [PubMed: 12039935]
42. Rowinsky EK, et al. Ras protein farnesyltransferase: A strategic target for anticancer therapeutic development. *J. Clin. Oncol* 1999;17:3631–3652. [PubMed: 10550163]
43. Emami KH, et al. A small molecule inhibitor of {beta}-catenin/CREB-binding protein transcription. *PNAS* 2004;101:12682–12687. [PubMed: 15314234]
44. Arber N, et al. Celecoxib for the prevention of colorectal adenomatous polyps. *N. Engl. J. Med* 2006;355:885–895. [PubMed: 16943401]
45. Baron JA, et al. A randomized trial of rofecoxib for the chemoprevention of colorectal adenomas. *Gastroenterology*. 2006 epub.
46. Bertagnolli MM, et al. Celecoxib for the prevention of sporadic colorectal adenomas. *N. Engl. J. Med* 2006;355:873–884. [PubMed: 16943400]
47. Gibson TB, et al. Randomized phase III trial results of panitumumab, a fully human anti-epidermal growth factor receptor monoclonal antibody, in metastatic colorectal cancer. *Clin. Colorectal. Cancer* 2006;6:29–31. [PubMed: 16796788]
48. Erlichman C, et al. Phase I study of EKB-569, an irreversible inhibitor of the epidermal growth factor receptor, in patients with advanced solid tumors. *J. Clin. Oncol* 2006;24:2252–2260. [PubMed: 16710023]
49. Attard G, et al. Phase I trial involving the pharmacodynamic study of circulating tumor cells, of CP-751,871, a monoclonal antibody against the insulin-like growth factor 1 receptor (IGF-1R), with docetaxel in patients with advanced cancer. *J. Clin. Oncol* 2006;24(18S):3023.
50. Crul M, et al. Phase I and pharmacological study of daily oral administration of perifosine (D-21266) in patients with advanced solid tumors. *Eur. J. Cancer* 2002;38:1615–1621. [PubMed: 12142051]

Figure 1.

Key mechanisms involved in colorectal carcinogenesis. The top half depicts key mutations that are required for progression along the adenoma–carcinoma axis in the chromosomal instability (CIN) pathway. One of the major mutational targets of *MUTYH* is the *APC* gene, the so-called gatekeeper to the adenoma–carcinoma axis. Progression along this axis is accompanied by corresponding mutations in genes such as *KRAS* and *TP53*. The bottom half depicts some of the key mutations in the microsatellite instability (MSI) pathway, *MLH1*, *MSH2*, and *MSH6*. The DNA mismatch repair gene *MLH1* can be inactivated either by a mutation or by promoter hypermethylation, which typically occurs in the context of the CpG island methylator phenotype (CIMP). *BRAF* mutations and *MLH1* hypermethylation are associated with an alternative form of polyp—serrated polyps. Abbreviations: LOH—loss of heterozygosity; APC—Adenomatous Polyposis Coli; COX2—cyclooxygenase 2; MLH1—mutL homolog 1; MSH2—mutS homolog 2; MSH6—mutS homolog 6; MYH—mutY homolog.

Figure 2.

DNA methylation of promoter CpG islands results in transcriptional silencing of key tumor suppressor genes, such as *MLH1*. DNA methyltransferases can transfer methyl groups from the carrier *S*-adenosine-methionine (SAM) to CpG dinucleotides. This is balanced by DNA demethylases that remove methyl groups from these CpG sequences. The mechanisms that regulate these two enzymatic processes are still unknown. Silencing of key tumor suppressor genes can lead to the formation of colorectal cancer.

Figure 3.

Loss of imprinting of the maternal insulin-like growth factor II (*IGF2*) allele predisposes to colorectal cancer. Imprinting of the maternal (M) allele (designated by the (*) in (a)) results in transcription of only the paternal (P) copy of *IGF2*. The imprinted region is indicated by the yellow oval. Loss of imprinting of the maternal allele (b) results in transcription from both the maternal and paternal copies of *IGF2*. This is associated with an increased risk for the development of colorectal cancer.

Targeted therapies in colorectal cancer

Table 1

Target	Strategic approach to target	Therapies in trial	Refs
K-ras	Inhibition of farnesylation of K-ras	Tipifarnib (Phase I/II trials in leukemia, pancreatic cancer, breast cancer, glioma), lonafarnib (Phase I/II trials in myelodysplastic syndrome, ovarian cancer, breast cancer).	[41,42] http://www.clinicaltrials.gov/
β-catenin/TCF-4^a	Inhibition of Wnt signaling by blocking interaction of β -catenin/TCF-4 with CBP ^b	ICG-001 in pre-clinical testing	[43]
COX2^c	Selective inhibition of COX2 enzymatic activity	Phase III trials of celecoxib, rofecoxib for chemoprevention complete. Multiple trials in combination with chemotherapy ongoing	[44–46]
EGFR^d	Inhibition of EGFR signaling with a monoclonal antibody	Cetuximab (FDA approved); panitumumab (Phase III complete)	[47]
	Inhibition of EGFR tyrosine kinase activity	Erlotinib (Phase I/II/III trials in combination with other agents); gefitinib (FDA approved for lung cancer); EKB-569 (Phase I complete)	[48]
VEGF^e	Inhibition of VEGF with a neutralizing antibody	Bevacizumab (FDA approved)	[39]
VEGFR^f	Inhibition of VEGFR tyrosine kinase to block angiogenesis	Sorafenib/BAY 43-9006, Vatalanib/PTK787/ZK222584 (Phase I/II)	http://www.clinicaltrials.gov/ NCT00171587 NCT00134069 NCT00326495
IGF1R^g	Inhibition of IGF1 receptor signaling with a specific antibody	CP-751,871 (Phase I/II trials for lung cancer, myeloma)	[49]
	Inhibition of IGF1R tyrosine kinase activity	INSM-18 (Phase I/II for prostate cancer)	http://www.insmed.com/
Raf	Inhibition of RAF kinase activity	Sorafenib/BAY 43-9006	http://www.clinicaltrials.gov/
MEK^h	Inhibition of MEK kinase activity	PD-325901 (Phase I/II)	http://www.clinicaltrials.gov/ NCT00147550
Akt	Inhibition of translocation to the membrane	Perifosine (Phase I/II)	[50]

^aT-cell factor 4.

^bCREB binding protein.

^cCyclooxygenase 2.

^dEpidermal growth factor receptor.

^eVascular endothelial growth factor.

f Vascular endothelial growth factor receptor.

g Insulin like growth factor 1 receptor.

h MAPK/ERK kinase.