Langenbeck's Arch Surg (1998) 383: 389–396

M. Hahn · O. N. Koufaki · H. K. Schackert

Molecular biology of colorectal cancer and clinical consequences for colorectal cancer syndromes

Received: 10 December 1997 / Accepted: 18 June 1998

Abstract Because of the accomplishments in biotechnical research in the past few decades our knowledge about the molecular mechanisms of carcinogenesis has grown rapidly. Colorectal cancer has been one of the most intensively investigated tumor entities, and it seems to be well established that colorectal tumor growth is associated with an accumulation of acquired somatic mutational events in tumor suppressor genes and oncogenes. Recent progress in our understanding of the molecular basis of the most prevalent colorectal cancer syndromes, such as hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP), is reflected by modifications in diagnosis and therapy. Identification and characterization of the causative genes for these colorectal cancer syndromes have enabled precise presymptomatic detection of mutations in individuals who bear an a priori risk of about 50% of developing colorectal cancer. Genotype-phenotype correlations might further increase the clinical management of hereditary colorectal cancer. Even though developments in cancer research are restricted to the minority of individuals with hereditary cancer syndromes, growing knowledge about the effect of low penetrance variations in tumor suppressor genes may affect the diagnosis and therapy of sporadic colorectal cancer.

Key words Cancer · Colorectal · Molecular · Hereditary · Diagnosis

Abbreviations FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colorectal cancer

M. Hahn (✉) · O. N. Koufaki · H. K. Schackert
Abteilung Chirurgische Forschung,
Universitätsklinikum Carl Gustav Carus,
Technische Universität Dresden,
Fetscherstrasse 74, D-01307 Dresden, Germany
Tel.: +49-351-458-3873, 3598; Fax: +49-351-458-4350

Introduction

Colorectal cancer is one of the most common cancers in Western populations, striking women and men at approximately equal rates. A genetic basis for the development of cancer was suggested by Karl Heinrich Bauer as early as 1928 [4], but only since the advancement of molecular biology has direct evidence been obtained to support the notion that cancer is a genetic disease. Molecular genetic studies have provided insights into the carcinogenesis of numerous tumors, and there is an especially large body of evidence that genetic changes accumulate during development of colorectal cancers [29]. Clinical screening to detect precancerous conditions or cancer at a curable stage is a means of reducing morbidity and mortality. In addition, identification of genetic risk factors for the development of adenomas and associated carcinomas of the colon and rectum enables predictive molecular diagnosis of malignant disease and thus might result in preventive surgical treatment.

Molecular aspects of colorectal tumorigenesis

There is growing evidence that both environmental [67] and genetic factors [29] contribute to colorectal tumorigenesis. Critical genetic factors in cancer development involve genes associated with cell regulation, cell proliferation, and differentiation, namely oncogenes, tumor suppressor genes, and caretaker genes [30].

Originally oncogenes were described as retroviral genes responsible for in vivo cell transformation and development of certain animal tumors (v-onc) and subsequently identified in normal human cells (proto-oncogenes) and in activated form in human tumors. Many of them play a physiological role in the regulation of cell division and differentiation. Therefore they are a common target for mutagenesis in neoplasia [3, 76]. Genes that code for growth-retarding products are inactivated by mutations in the

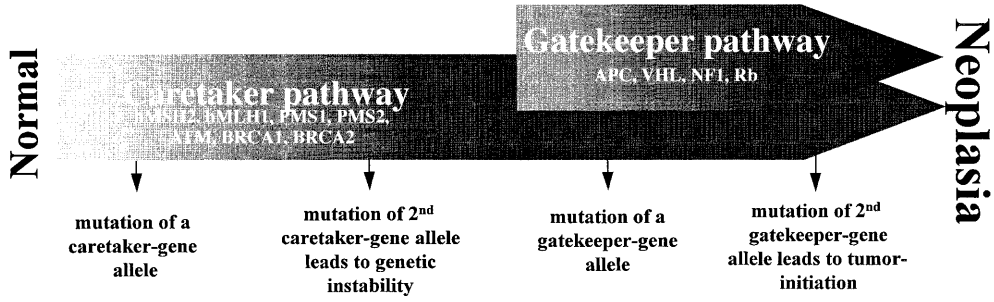

Fig. 1 Pathways to neoplasia. Both inherited caretaker and gatekeeper mutations predispose to tumor development. While one additional mutation of the second gatekeeper gene allele may be sufficient for tumor initiation in the gatekeeper pathway, a caretaker gene alteration requires three additional genetic changes, namely mutation of the second caretaker gene allele and, as a result of genetic instability, alterations in gatekeeper genes. (Modified from [30])

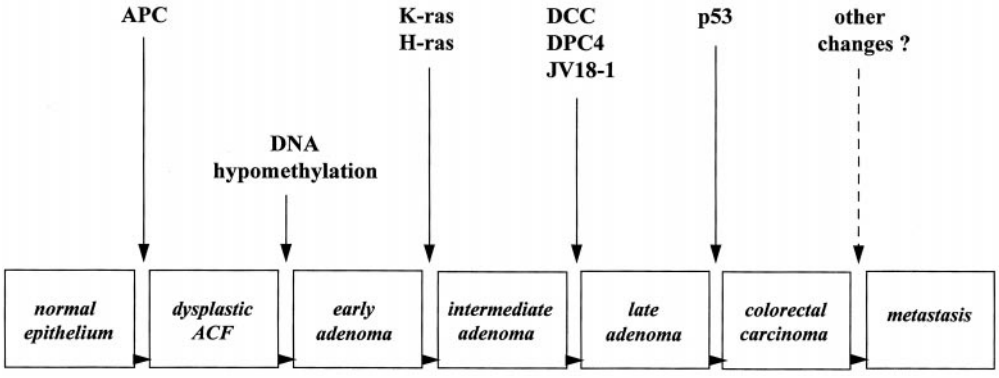

Fig. 2 Genetic model of colorectal tumorigenesis. (Modified from [29])

course of tumorigenesis and are thus called tumor suppressor genes. These are at least as important as oncogenes in the development of tumor. Their loss, as a germline or as a somatic mutation, may contribute to cancer. The existence of these kinds of genes has been suspected in cell hybrids between transformed and normal cells, the transformed phenotype being recessive, which reappears after substantial chromosome loss [32]. In contrast, caretaker gene inactivation does not lead directly to tumor development. Because of increased mutation rates resulting from inactivation of both alleles of caretaker genes, tumor suppressor genes are targeted and inactivated and therefore result in tumor initiation (Fig. 1) [29, 30].

Involvement of oncogenes and tumor suppressor genes in tumorigenesis of colorectal tumors

Somatic gene alterations are present at various stages of colorectal tumor formation. In 1990 Fearon and Vogelstein [18] proposed a model of carcinogenesis on the basis of molecular alterations in DNA (Fig. 2). This model describes the potential sequence of genetic alterations providing these cells with a growth advantage and as a consequence constitute the predominant cell type within the tumor (clonal expansion). The initial step in the majority of all colorectal carcinomas may be the mutation of the *APC* tumor suppressor gene in one colon mucosa cell. As a result the cell divides repeatedly, initiating a cell clone that forms a mucosal hyperplasia. Subsequently, epigenetic events such as a change in the methylation status of DNA occur, thus predisposing to additional mutations. *Ras* oncogene mutations usually occur in one cell of a preexisting adenoma and may be detected during conversion of a small adenoma to a larger dysplastic one. Further mutations follow, and a mutation in the tumor suppressor gene *p53*, accompanied by deletion of the second allele [32], is frequently observed at the transition from adenoma with severe cell dysplasia to invasive carcinoma. This cell clone obviously has the capacity for invasive growth and is therefore defined as a carcinoma.

Additional genetic changes are required to allow single cells to separate from a tumor, spread to other organs, and generate metastasis. The sequence of mutations presented here may vary substantially in different colorectal tumors. However, mutations in the *APC* [60] and *ras* genes [76] are obviously frequent early events of the adenoma to carcinoma sequence. Likewise, a mutation/deletion in the *p53* gene is frequently encountered at the transition from adenoma to carcinoma. The basic principle is the accumulation of oncogene activations and tumor suppressor gene mutations and loss of the second alleles (loss of heterozygosity, LOH) in these tumor suppressor genes [18]. This model of carcinogenesis is probably valid for the vast majority of colorectal carcinomas.

Involvement of caretaker genes in tumorigenesis of colorectal tumors

Mutations in five identified human genes are associated with hereditary predisposition to nonpolyposis colorectal

cancer (HNPCC). These genes, subsumed under mismatch repair genes, are human homologues of yeast and bacterial DNA-repair genes and encompass the genes *hMSH2* on chromosome 2p [19, 34], *hMLH1* on chromosome 3p [9, 52], *hPMS1* on chromosome 2q, and *hPMS2* on chromosome 7p [49]. *GTBP*, also called *hMSH6* [16, 51, 53], has rarely been found to be associated with HNPCC [42, 53]. Genomic DNA in HNPCC tumors is characterized by instability in repetitive DNA sequences (microsatellites), which reflect replication errors of the DNA polymerase that cannot be repaired because of mutational inactivation of at least one mismatch repair gene [1, 55, 66]. Sporadic tumors featuring microsatellite instabilities due to sporadic mutational inactivation of a mismatch repair gene are localized primarily in the right colon; they are frequently diploid and show an inverse relationship to *p53* mutations – features that are in common with HNPCC-associated colorectal tumors [25, 28]. These data suggest another pathway to neoplasia in addition to the accumulation of oncogene and tumor suppressor gene mutations as described above [11, 30]. High mutation rates in HNPCC-associated tumors due to defects in DNA repair genes may affect tumor suppressor genes and lead to tumor development [30, 39, 61].

Hereditary colorectal cancer syndromes

Based on the models presented above, colorectal tumorigenesis may result either from inherited mutations or from spontaneous mutations in tumor suppressor genes or caretaker genes.

Familial adenomatous polyposis

One of the most extensively studied inherited cancer syndromes is familial adenomatous polyposis (FAP), a genetically determined disease that typically presents with extensive adenomatous polyps of the colon in early adult life. Polyposis is a premalignant disease with a probability of almost 100% that one or more polyps will progress through dysplasia to malignancy in untreated gene carriers. Occasionally the extracolonic features of the syndrome lead to diagnosis. In the past early detection of this cancer predisposition syndrome at a presymptomatic stage was feasible only by linkage analyses on chromosome 5q21 and was restricted to FAP families with at least two affected members. In 1991 the *APC* gene was identified and characterized, and *APC* germline mutations have been detected in FAP families [23, 27, 31, 50]. In contrast to the requirements for linkage analysis, sequence analysis of the gene can identify unrelated gene-mutation carriers. At least 332 germline mutations have been reported thus far [7], with the most frequent mutations occurring in exon 15 at codons 1309 and 1061 [7, 38, 46]. More than one-half of all *APC* mutations are localized within a region of exon 15, encompassing 600 codons [43, 48]. An *APC* mutation database [7] will provide data on the kind of genetic alterations and their distribution, which will further improve the general *APC* mutation detection strategy. It is estimated that a mutation detection rate of 90% can be achieved [14].

Hereditary nonpolyposis colorectal cancer

FAP accounts for approximately 1% of all colorectal cancers. Unlike FAP, the HNPCC syndrome, originally termed Lynch syndrome, is associated with germline mutations in at least five mismatch repair genes that are believed to cause up to about 5% of all colorectal carcinomas [2, 40, 41, 58] and a variety of extracolonic tumors [6, 62]. Because there is lack of evident clinical markers, such as a manifest polyposis in FAP, a familial history, referred to as the Amsterdam criteria, is the cornerstone for the identification of affected families. The lifetime risk of mutation gene carriers for developing colorectal carcinoma is estimated to be 80–90% [75], which implies rigid clinical screening activities in HNPCC families. According to the guidelines proposed by the international collaborative group on HNPCC in 1996, a regular colonoscopic examination should be performed for all individuals at risk in HNPCC families [77]. It has been shown that a clinical surveillance program in HNPCC families decreases the morbidity rate by about 62% [26].

Genotype-phenotype correlations

Analyses of the *APC* gene have revealed that mutations in a region between codons 1250 and 1464 in exon 15 are associated with a severe, early-onset polyposis in the colon and rectum [12, 47]. On the other hand, late onset and an attenuated type of FAP are correlated with mutations from the 5’ end through exon 4 [64]. Subsequent studies have suggested that a second region within exon 15 around codon 1597 is responsible for the attenuated phenotype [20]. A functional boundary for the congenital hypertrophy of the retinal pigment epithelium has been localized in exon 9, with downstream mutations associated with this phenotypical feature. An exception to this rule seems to be a region in exon 15 between codons 1445 and 1578, where mutations are linked with normal retina. Mutations in this defined region, however, are correlated with the development of desmoid tumors in all affected families [13]. Clear genotype-phenotype correlations, as observed in FAP, have not yet been found in the HNPCC syndrome, which may be because of the low number of HNPCC families analyzed so far.

General knowledge on genotype-phenotype correlations in combination with individual genotype analysis may contribute to a clear prediction about the course of the disease and hence substantially influence both the individual strategy of surveillance and individual preventive therapy. On the other hand, it is conceivable that knowledge about the individual phenotype would streamline molecular diagnosis by indicating the localization of the causative mutation. Nevertheless, it must be taken into consid-

eration that exogenous factors or additional genes may play a key role in FAP development since identical *APC* mutations may result in varying phenotypes [24, 50, 54]. In accordance with a gene locus on chromosome 4 affecting the expression of polyposis in mice [15], there are probably modifying genes in humans that are responsible for phenotype variations [65].

Implications for diagnosis and treatment of colorectal tumor

Preliminary considerations

The genetic model of carcinogenesis implies consequences for both the diagnosis and therapy of malignant tumors. Individuals who are exposed to endogenous (genetic) and exogenous (environmental) factors may be identified and subjected to a surveillance program and/or preventive surgical intervention against tumor development. Correlation of genotype and phenotype in individuals at risk is essential for the development of a specific preventive/therapeutic approach in mutation carriers, while non-mutation carriers – provided that the causative mutation has previously been identified in mutation carriers of that family – can be released from the colonoscopic surveillance program. If gene mutations are the cause of tumor development, tumors might possibly be treated by causative gene therapy transferring wild-type gene copies into tumor cells. Furthermore, correlation of the molecular cause (genotype) and clinical expression (phenotype) of the disease form the basis of the molecular nosology of malignancies.

FAP

Because of the autosomal dominant mode of inheritance, first-degree relatives of affected family members are at an a priori risk of 50% of being mutation carriers who will develop polyposis and, as a consequence, colorectal cancer with high probability. Family members at risk whose mutation carrier status is unknown should thus be offered annual flexible sigmoidoscopy examinations beginning around puberty [78]. The objective of this screening is early detection of a manifest polyposis prior to cancer development in individuals who have inherited the gene defect [44]. On average, one-half of relatives in FAP families are non-mutation carriers with no elevated colorectal cancer risk compared to the normal population. However, without molecular diagnosis these individuals must undergo unnecessary regular endoscopic screening without clinical consequences.

A growing number of commercial and university-based laboratories are offering genetic testing for hereditary colorectal cancer syndromes. A set of molecular techniques allow accurate presymptomatic determination of the mutation carrier status of individuals at risk in a high proportion of FAP families prior to adenoma development. Most reliable genetic test results can be accomplished by DNA sequencing with or without a molecular prescreening approach such as single-strand conformation polymorphism (SSCP) [71], denaturing gradient gel electrophoresis (DGGE) [69], and the protein truncation test (PTT) [59, 68, 69]. These genetic tests should not be offered before puberty [78]. Because a negative or positive test result may have considerable medical and psychological significance, all individuals who are at risk of developing hereditary colorectal cancer should undergo pre- and posttest genetic counseling. In any case, their willingness to undergo genetic testing should be documented by written informed consent.

Clinical consequences of a negative genetic test result

A negative genetic test result in an at risk individual rules out FAP only if the disease causing germline mutation can be identified in at least one affected FAP family member. If this mutation cannot be found, no reliable statement of individual mutation carrier status is possible. If non-mutation carriers can be precisely identified prior to onset of disease, colon screening of non-mutation carriers can be reduced to three time points – 18, 25, and 35 years [56] – or, if long-term clinical data confirm the accuracy of molecular testing in the future, they can be completely discharged from colorectal screening. Presymptomatic genetic testing implies a series of impressive advantages. In contrast to endoscopic procedures a noninvasive genetic test must be performed only once, involving no physical complications. A negative test result relieves non-mutation carriers from the distress of having an elevated colorectal cancer risk. In addition, it might enhance compliance with conventional endoscopic examinations in those whose test result is positive. There should be no concerns with regard to increased costs due to molecular testing, as regular endoscopic examinations of non-mutation carriers are in general more expensive than one-time molecular DNA sequencing for all individuals at risk in a large FAP family. When the specific *APC* gene mutation has once been identified in an individual FAP family, a rapid, straightforward, and inexpensive DNA sequence analysis may be offered to all relatives in that family. It should be noted that non-mutation carriers bear almost the same risk of developing a spontaneous colorectal tumor as the general population and should undergo the routine cancer-screening programs offered to the public.

Clinical consequences of a positive test result

No change in conventional screening regimen is recommended for those whose presymptomatic DNA analysis indicates that they have inherited the disease-causing *APC* allele [56]. The essential posttest genetic counseling described above is of special significance in these patients because FAP mutation carriers must deal with the high risk and the prospects of having to undergo the single preven-

tive strategy, restorative proctocolectomy. Colectomy should be carried out as soon as polyposis is present, but not before adolescence, with the exception of severe symptoms caused by massive polyposis in childhood [13]. Because of preliminary data on genotype/phenotype correlations, Vasen et al. [74] have asked whether subtotal colectomy and ileorectal anastomosis rather than restorative proctocolectomy should be the primary treatment for polyposis in patients with mutations upstream from codon 1250. Their line of argument is based on the fact that *APC* gene carriers with mutations downstream from codon 1250 in exon 15, especially at codon 1309, tend to develop a more aggressive course of disease and have a higher risk of rectal excision due to more frequent metachronous tumor development. This example illustrates how data on genotype/phenotype correlations may contribute to the individual clinical decision-making process.

HNPCC

HNPCC shows a pattern of inheritance similar to that of FAP, but it is more difficult to diagnose clinically because it does not have an obvious and universal phenotypic marker, such as the colorectal polyposis of FAP. Therefore data on familial history are generally used to identify families at risk. The Amsterdam criteria require at least three affected family members, one of whom is a first-degree relative of the other two. Colorectal carcinoma develops in at least two generations with at least one family member younger than 50 years of age at the time of colorectal cancer diagnosis [72].

Because tumors in HNPCC affect predominantly the right colon, an adequate HNPCC surveillance program requires regular total colonoscopic examinations. Individuals at risk should be offered colonoscopy every 1–2 years, starting between 20 and 30 years of age, and every year after age 40 years [78]. Due to the extracolonic features of HNPCC, regular gynecological examinations are recommended for endometrial cancer, including transvaginal sonography and CA-125 measurement. Additional screening procedures, such as gastroscopy and examinations of the urinary tract, should be performed depending on the family history [77].

Direct gene analysis by DNA sequencing alone or in combination with screening techniques for rapid identification of such mutations are currently being used successfully, as demonstrated by a number of studies [10, 33, 35, 36, 45]. As with FAP, genetic testing in HNPCC presupposes genetic counseling prior to and after molecular analysis.

Clinical consequences of a negative test result

Non-mutation carriers may be discharged from further lifelong colonoscopic surveillance if the functionally relevant mutation in each individual HNPCC family has been precisely detected and characterized [5, 22, 26, 57, 70, 73]. As in FAP, persons at risk of HNPCC in families in which the disease-causing mutation has been identified, but who are non-mutation carriers, are relieved from life-long clinical screening for HNPCC associated tumors. The risk of developing a spontaneous colorectal tumor is similar to that in the general population and requires the screening programs offered to the public. In addition to the undeniable psychological benefits, genetic testing even provides a saving in terms of costs.

Clinical consequences of a positive test result

Individuals who test positive face an elevated risk of developing colorectal cancer. The lack of a clear phenotypic marker, such as polyposis in FAP and the accelerated conversion rate from adenoma to carcinoma [73], raise the question of whether there is a role for prophylactic subtotal colectomy among HNPCC germline mutation carriers [37]. Vasen et al. [75] have estimated the risk of developing carcinoma in a 40-year old male mutation carrier at 31.5% over a period of 35 years despite regular clinical screening using colonoscopy alone or sigmoidoscopy in combination with barium enema and regular removal of all developed polyps. These data would clearly favor prophylactic colectomy as a primary intervention in gene mutation carriers.

On the other hand, the life-time risk for colorectal carcinoma in HNPCC gene mutation carriers is difficult to assess, but it is clearly less than 100% and is still under discussion [17, 75]. Without exact data individual therapeutic decisions in terms of prophylactic surgical interventions are difficult to make. Furthermore, despite prophylactic colectomy endoscopic explorations of the remaining rectum must be performed at regular intervals since the risk of rectal cancer is estimated at 3% every 3 years for the first 12 years after colectomy [63]. Female carriers of the mismatch repair gene mutation face an elevated risk of endometrial cancer [17, 75], that would imply the necessity for a prophylactic hysterectomy. In addition, a vast number of prophylactic surgical interventions are conceivable to rule out all extracolonic cancer risks associated with HNPCC. All these considerations might undermine the benefit derived from prophylactic colectomy.

In conclusion, the benefits and disadvantages of prophylactic surgery must be investigated in controlled clinical studies on the basis of the identification of the disease-causing gene mutation. Clinical implications should be discussed on an individual basis, with special consideration to family history, compliance with a colonoscopic surveillance program, and the social background of the individual mutation gene carrier. All gene carriers should be informed about the entire range of available diagnostic and therapeutic options before they make their final decision.

Diagnostic and therapeutic achievements of genetic testing are generally restricted to individuals affected by hereditary cancer syndromes. The majority of the population bear no generally elevated risk of colorectal cancer. However, since cancer is basically a genetic disease, the

risk of cancer varies between individuals. It is therefore tempting to assess the role of low-penetrance germline variations that might enhance individual cancer risk without being apparent in the family history. There is a growing body of evidence that low-penetrance gene variations predispose to cancer [8, 21]. Further research will show the significance of this aspect for predictive diagnosis in conjunction with preventive approaches for "sporadic" colorectal cancer.

References

1. Aaltonen LA, Peltomäki P, Leach FS, Sistonen P, Pylkkänen L, Mecklin JP, Järvinen H, Powell SM, Jen J, Hamilton SR, Petersen GM, Kinzler KW, Vogelstein B, de la Chapelle A (1993) Clues to the pathogenesis of familial colorectal cancer. Science 260:812–816
2. Altonen LA, Sankila R, Mecklin J-P, Järvinen H, Pukkala E, Peltomaki P, de la Capelle A (1994) A novel approach to estimate the proportion of hereditary nonpolyposis colorectal cancer of total colorectal cancer burden. Cancer Detect Prevent 18:57–63
3. Barbacid M (1987) ras genes. Annu Rev Biochem 56:779–827
4. Bauer KH (1928) Mutationstheorie der Geschwulst-Entstehung. Übergang von Körperzellen in Geschwulstzellen durch Gen-Änderung. Springer, Berlin
5. Bellacosa A, Genuardi M, Anti M, Viel A, Ponz de Leon M (1996) Hereditary nonpolyposis colorectal cancer: review of clinical, molecular genetics, and counseling aspects Am J Med Genet 62:353–364
6. Benatti P, Sassatelli R, Roncucci L, Pedroni M, Fante R, Di Gregorio C, Losi L, Gelmini R, Ponz de Leon M (1993) Tumour spectrum in heredirary non-polyposis colorectal cancer (HNPCC) and in families with "suspected HNPCC." A population-based study in Northern Italy. Int J Cancer 54:371–377
7. Beroud C, Soussi T (1996) APC gene: database of germline and somatic mutations in human tumors and cell lines. Nucleic Acids Res 24:121–124
8. Brentnall TA, Rubin CE, Crispin DA, Stevens A, Batchelor RH, Haggitt RC, Bronner MP, Evans JP, McCahill LE, Bilir N, Boland CR, Rabinovitch PS (1995) A germline substitution in the human MSH2 gene is associated with high-grade dysplasia and cancer in ulcerative colitis. Gastroenterology 109:151–155
9. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergard P, Bollag RJ, Godwin AR, Ward DC, Nordenskjold M, Fishel R, Kolodner R, Liskay RM (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368:258–261
10. Buerstedde JM, Alday P, Torhorst J, Weber W, Muller H, Scott R (1995) Detection of new mutations in six out of 10 Swiss HNPCC families by genomic sequencing of the hMSH2 and hMLH1 genes. J Med Genet 32:909–912
11. Carder P, Wyllie AH, Purdie CA, Morris RG, White S, Piris J, Bird CC (1993) Stabilized p53 facilitates aneuploid clonal divergence in colorectal cancer. Oncogene 8:1397–1401
12. Caspari R, Friedl W, Mandl M, Möslein G, Kadmon M, Knapp M, Jacobasch K.-H, Ecker K-W, Kreissler-Haag D, Timmermanns G, Propping P (1994) Familial adenomatous polyposis: mutation at codon 1309 and early onset of colon cancer. Lancet 343:629–632
13. Caspari R, Olschwang S, Friedl W, Mandl M, Boisson C, Boker T, Augustin A, Kadmon M, Möslein G, Thomas G, Propping P (1995) Familial adenomatous polyposis: desmoid tumours and lack of ophthalmic lesions (CHRPE) associated with APC mutations beyond codon 1444. Hum Mol Genet 4:337–340
14. Cunningham D, Dunlop MG (1996) Molecular genetic basis of colorectal cancer susceptibility. Br J Surg 83:321–329
15. Dietrich WF, Lander ES, Smith JS, Moser AR, Gould KA, Luongo C, Borenstein N, Dove W (1993) Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. Cell 75:631–639
16. Drummond JT, Li G-M, Longley MJ, Modrich P (1995) Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science 268:1909–1912
17. Dunlop MG, Farrington SM, Carothers AD, Wyllie AH, Sharp L, Burn J, Liu B, Kinzler KW, Vogelstein B (1997) Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 6:105–110
18. Fearon ER, Vogelstein B (1990) A genetic model for colorectal tumorigenesis. Cell 61:759–767
19. Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75:1027–1038
20. Friedl W, Meuschel, S, Caspari R, Lamberti C, Krieger S, Sengteller M, Propping P (1996) Attenuated familial adenomatous polyposis due to a mutation in the 3-prime part of the APC gene: a clue for understanding the function of the APC protein. Hum Genet 97:579–584
21. Goessl C, Plaschke J, Pistorius S, Frank S, Hampl M, Görgens H, Koch R, Saeger H-D, Schackert HK (1998) An intronic germline transition in the HNPCC gene hMSH2 is associated with sporadic colorectal cancer in a german population. Eur J Cancer (in press)
22. Green SE, Chapman PD, Burn J, Bishop DT, Varma JS (1995) Clinical impact of colonoscopic screening in first-degree relatives of patients with hereditary non-polyposis colorectal cancer. Br J Surg 82:1338–1340
23. Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H, Joslyn G, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R (1991) Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66:589–600
24. Groden J, Gelbert L, Thliveris A, Nelson L, Robertson M, Joslyn G, Samowitz W, Spirio L, Carlson M, Burt R, Leppert M, White R (1993) Mutational analysis of patients with adenomatous polyposis: identical inactivating mutations in unrelated individuals. Am J Hum Genet 52:263–272
25. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M (1993) Ubiquitious somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 363:558–561
26. Järvinen HJ, Jukka-Pekka M, Sistonen P (1995) Screening reduces colorectal cancer rate in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 108:1405–1411
27. Joslyn G, Carlson M, Thliveris A, Albertsen H, Gelbert L, Samowitz W, Groden J, Stevens J, Spirio L, Robertson M, Sargeant L, Krapcho K, Wolff E, Burt R, Hughes JP, Warrington J, McPherson J, Wasmuth J, Le Paslier D, Abderrahim H, Cohen D, Leppert M, White R (1991) Identification of deletion mutations and three new genes at the familial polyposis locus. Cell 66:601–613
28. Kim H, Jen J, Vogelstein B, Hamilton SR (1994) Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. Am J Pathol 145:148–156
29. Kinzler KW, Vogelstein B (1996) Lessons from hereditary colorectal cancer. Cell 87:159–170
30. Kinzler KW, Vogelstein B (1997) Gatekeepers and caretakers. Nature 386:761–763
31. Kinzler KW, Nilbert, MC, Su L-K, Vogelstein B, Bryan TM, Levy DB, Smith KJ, Preisinger AC, Hedge P, McKechnie D, Finniear R, Markham A, Groffen J, Boguski MS, Altschul SF, Horii A, Ando H, Miyoshi Y, Miki Y, Nishisho I, Nakamura Y (1991) Identification of FAP locus genes from chromosome 5q21. Science 253:661–665
32. Knudson AG (1996) Hereditary cancer: two hits revisited. J Cancer Res Clin Oncol 122:135–140

33. Kohonen-Corish M, Ross VL, Doe WF, Kool DA, Edkins E, Faragher I, Wijnen J, Khan PM, Macrae F, St John DJB (1996) RNA-based mutation screening in hereditary nonpolyposis colorectal cancer. Am J Hum Genet 59:818–824
34. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jean J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom-Lahti M, Guan XY, Zhang J, Meltzer PS, Yu J-W, Kao F-T, Chen DJ, Cerosaletti KM, Fournier REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin J-P, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch HT, Trent JM, de la Chapelle A, Kinzler KW, Vogelstein B (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75:1215–1225
35. Liu B, Parsons N, Papadopoulos N, Nicolaides NC, Lynch HT, Watson P, Jass JR, Dunlop M, Wyllie A, Peltomaki P, de la Capelle A, Hamilton SR, Vogelstein B, Kinzler KW (1996) Analysis of mismatch repair genes in hereditary non-plyposis colorectal cancer patients. 2:169–174
36. Luce MC, Marra G, Chauhan DP, Laghi L, Carethers JM, Cherian SP, Hawn M, Binnie CG, Kam-Morgan LNW, Cayouette MC, Koi M, Boland CR (1995) In vitro transcription/translation assay for the screening of hMLH1 and hMSH2 mutations in familial colon cancer. Gastroenterology 109:1368–1374
37. Lynch HT (1996) Is there a role for prophylactic subtotal colectomy among hereditary nonpolyposis colorectal cancer germline mutation carriers? Dis Colon Rectum 39:109–110
38. Mandl M, Paffenholz R, Friedl W, Caspari R, Sengteller M, Propping P (1994) Frequency of common and novel inactivating APC muations in 202 families with familial adenomatous polyposis. Hum Mol Genet 3:181–184
39. Markowitz, S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B (1995) Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 268:1336–1338
40. Mecklin JP (1987) Frequency of hereditary colorectal carcinoma. Gastroenterology 93:1021–102539
41. Mecklin JP, Ponz-de-Leon M (1994) Epidemiology of HNPCC. Anticancer Res 14:1625–1629
42. Miyaki M, Konishi M, Tanaka K, Kikuchi-Yanoshita R, Muraoka M, Yasuno M, Igari T, Koike M, Chiba M, Mori T (1997) Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 17:271–272
43. Miyoshi Y, Ando H, Nagase H, Nishisho I, Horii A, Miki Y, Mori T, Utsunomiya J, Baba S, Petersen G, Hamilton SR, Kinzler KW, Vogelstein B, Nakamura Y (1992) Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. Proc Natl Acad Sci USA 89:4452–4456
44. Möslein G, Buhr HJ, Kadmon M, Herfarth C (1992) Familiäre adenomatöse Polyposis. Chirurg 63:327–333
45. Möslein G, Tester DJ, Lindor NM, Honchel R, Cunningham JM, French AJ, Halling KC, Schwab M, Goretzki P, Thibodeau SN (1996) Microsatellite instability and mutation analysis of hMSH2 and hMLH1 in patients with sporadic, familial and hereditary colorectal cancer. Cancer Res 5:1245–1252
46. Nagase H, Nakamura Y (1993) Muations of the APC (adenomatous polyposis coli) gene. Hum Mutat 2:425–434
47. Nagase H, Miyoshi Y, Horii A, Aoki T, Ogawa M, Utsunomiya J, Baba S, Sasazuki T, Nakamura Y (1992) Correlation between the location of germ-line mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis patients. Cancer Res 52:4055–4057
48. Nagase H, Miyoshi Y, Horii A, Aoki T, Petersen GM, Vogelstein B, Maher E, Ogawa M, Maruyama M, Utsunomiya J, Baba S, Nakamura Y (1992) Screening for germ-line mutations in familial adenomatous polyposis patients: 61 new patients and a summary of 150 unrelated patients. Hum Mutat 1:467–473
49. Nicolaides NC, Papadopulos N, Liu B, Wie Y-F, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Dunlop MG, Hamilton SR, Petersen GM, de la Capelle A, Vogelstein B, Kinzler KW (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371:75–80
50. Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A, Koyama K, Utsunomiya J, Baba S, Hedge P, Markham A, Krush AJ, Petersen G, Hamilton SR, Nilbert MC, Levy DB, Bryan TM, Preisinger AC, Smith KJ, Su L-K, Kinzler KW, Vogelstein B (1991) Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 253:665–669
51. Palombo F, Gallinari P, Iaccarino I, Lettieri T, Hughes M, Dárrigo A, Truong O, Hsuan JJ, Jiricny J (1995) GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. Science 268:1912–1914
52. Papadopoulos N, Nicolaides NC, Wei Y-F, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomaki P, Mecklin J-P, de la Chapelle A, Kinzler KW, Vogelstein B (1994) Mutation of a mutL homolog in hereditary colon cancer. Science 263:1625–1629
53. Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, D'Arrigo A, Markowitz S, Willson JKV, Kinzler KW, Jiricny J, Vogelstein B (1995) Mutations of GTBP in genetically unstable cells. Science 268:1915–1917
54. Paul P, Letteboer T, Gelbert L, Groden J, White R, Coppes MJ (1993) Identical APC exon 15 mutations result in a variable phenotype in familial adenomatous polyposis. Hum Mol Genet 2:925–931
55. Peltomaki P, Aaltonen LA, Sistonen P, Pylkkänen L, Mecklin J-P, Järvinen H, Green JS, Jass JR, Weber JL, Leach FS, Peterson GM, Hamilton SR, de la Chapelle A, Vogelstein B (1993) Genetic mapping of a locus predisposing to human colorectal cancer. Science 260:810–812
56. Petersen, GM, Francomano C, Kinzler K, Nakamura Y (1993) Presymptomatic direct detection of adenomatous polyposis coli (APC) gene mutations in familial adenomatous polyposis. Hum Genet 91:307–311
57. Piepoli A, Santoro R, Cristofaro G, Traversa GP, Gennarelli M, Accadia L, Siena D, Bisceglia M, Lynch HT, Peltomaki P, Andriulli A (1996) Linkage analysis identifies gene carriers among members of families with hereditary nonpolyposis colorectal cancer. Gastroenterology 110:1404–1409
58. Ponz de Leon M (1994) Prevalence of hereditary nonpolyposis colorectal carcinoma (HNPCC). Ann Med 26:209–214
59. Powell SM, Petersen GM, Krush AJ, Booker S, Jen J, Giardiello FM, Hamilton SR, Vogelstein B, Kinzler KW (1993) Molecular diagnosis of familial adenomatous polyposis. N Engl J Med 329:1982–1987
60. Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW (1992) APC mutations occur early during colorectal tumorigenesis. Nature 359:235–237
61. Rampino N, Yamamoto H, Ionov Y, Li Y, Swai H, Reed JC, Perucho M (1997) Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 275:967–969
62. Rodriguez-Bigas MA, Lee PHU, O'Malley L, Weber TK, Suh O, Anderson GR, Petrelli NJ (1996) Establishment of a hereditary nonpolyposis colorectal cancer registry. Dis Colon Rectum 39:649–653
63. Rodriguez-Bigas MA, Vasen HF, Pekka-Mecklin J, Myrhoj T, Rozen P, Bertario L, Järvinen HJ, Jass JR, Kunitomo K, Nomizu T, Driscoll DL (1997) Rectal cancer risk in hereditary nonpolyposis colorectal cancer after abdominal colectomy. International Collaborative Group on HNPCC. Ann Surg 225:202–207
64. Spirio L, Olschwang S, Groden J, Robertson M, Samowitz W, Joslyn G, Gelbert L, Thliveris A, Carlson M, Otterud B, Lynch H, Watson P, Lynch P, Laurent-Puig P, Burt R, Hughes JP, Thomas G, Leppert M White R (1993) Alleles of the APC gene: an attenuated form of familial polyposis. Cell 75:951–957
65. Spirio L, Kutchera W, Winstead M, Pearson B, Kaplan C, Robertson M, Lawrence E, Burt R, Tischfield B, Leppert M, Prescott S, White R (1996) Three secretory phospholipase A2 genes that map to human chromosome 1p35–36 are not mutated in individuals with attenuated adenomatous polyposis coli. Cancer Res 56:955–958

66. Thibodeau SN, Bren G, Schaid D (1993) Microsatellite instability in cancer of the proximal colon. Science 260:816–819
67. Thomas DB, Karagas MR (1987) Cancer in first and second generation Americans. Cancer Res 47:5771
68. Van der Luijt, RB, Khan PM, Vasen H, van Leeuwen C, Tops C, Roest P, den Dunnen J, Fodde R (1994) Rapid detection of translation-terminating mutations at the adenomatous polyposis coli (APC) gene by direct protein truncation test. Genomics 20:1–4
69. Van der Luijt RB, Khan PM, Vasen HFA, Tops CMJ, van Leeuwen-Cornelisse ISJ, Wijnen JT, van der Klift HM, Plug RJ, Griffioen G, Fodde R (1997) Molecular analysis of the APC gene in 105 Dutch kindreds with familial adenomatous polyposis: 67 germline mutations identified by DGGE, PTT, and Southern analysis. Hum Mutat 9:7–16
70. Van de Water NS, Jeevaratanam P, Browett PJ, Stewart SM, Lane MR, Jass JR (1994) Direct mutational analysis in a family with hereditary non-polyposis colorectal cancer. Aust NZ J Med 24:682–686
71. Varesco L, Gismondi V, James R, Robertson M, Grammatico P, Groden J, Casarino L, De Benedetti L, Bafico A, Bertario L, Sala P, Sassatelli R, Ponz de Leon M, Biasco G, Allegretti A, Aste H, De Sanctis S, Rossetti C, Illeni MT, Sciarra A, Del Porto G, White R, Ferrara GB (1993) Identification of APC gene mutations in Italian adenomatous polyposis coli patients by PCR-SSCP analysis. Am J Hum Genet 52:280–285
72. Vasen HFA, Mecklin JP Meera-Khan P, Lynch HT (1991) The International Collaborative Group on Hereditary Non-polyposis Colorectal Cancer. Dis Colon Rectum 34:424
73. Vasen HFA, Nagengast FM, Meera Khan P (1995) Interval cancers in hereditary non-polyposis colorectal cancer (Lynch syndrome). Lancet 345:1183–1184
74. Vasen HFA, van der Liijt RB, Slors JFM, Buskens E, deRuiter P, Baeten GM, Schouten WR, Oostvogel HJM, Kuijpers JHC, Tops CM, Meera Khan P (1996) Molecular genetics test as a guide to surgical managment of familial adenomatous polyposis. Lancet 348:433–435
75. Vasen HFA, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, Nagengast FM, Meijers-Heijboer EH, Bertario L, Varesco L, Bisgaard M-L, Mohr J, Fodde R, Meera-Khan P (1996) Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology 110:1020–1027
76. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL (1988) Genetic alterations during colorectal-tumor development. N Engl J Med 319:525
77. Weber T (1996) Clinical surveillance recommendations adopted for HNPCC. Lancet 348:465
78. Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, Murlow CD, Woolf SH, Glick SN, Ganiatis TG, Bond JH, Rosen L, Zapka JG, Olsen SJ, Giardiello FM, Sisk JE, van Antwerp R, Brown-Davis C, Marciniak DA, Mayer RJ (1997) Colorectal cancer screening: clinical guidelines and rationale. Gastroenterology 112:594–642