Molecular discrimination of sessile rectal adenomas from carcinomas for a better treatment choice: integration of chromosomal instability patterns and expression array analysis

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CHAPTER 1

General introduction

Rectal cancer

Epidemiology, incidence of CRC

Colorectal cancer (CRC) is one of the leading causes of death and accounts for approximately 300,000 new cases per year in Europe and the USA (1). Approximately 25% of these cases are rectal cancers. However, the incidence of adenomas, the benign precursors of carcinomas, is far higher; by the age of 70, approximately 50% of the western population will have developed an adenoma (2). About one in ten of these adenomas will ultimately progress to cancer, leading to a population risk of approximately 5% (3). Based on these figures, colorectal cancer is the third most common cancer in the western world.

Pathology

The normal colorectum consists of several distinct tissue layers: the mucosa, muscularis mucosae, submucosa, muscularis propria, subserosal fat and serosa, although in the rectum a serosal layer is absent (Figure 1). Through a series of well-defined histopathological changes, colorectal cancer develops from an adenoma into a carcinoma. Adenomas are intramucosal neoplasms and can be stratified according to architectural characteristics: tubular, tubulovillous, and villous (4). Furthermore, adenomas can be flat, pedunculated, or sessile. Most tubular adenomas are small and pedunculated, and most villous adenomas are large and sessile. The risk of progression to a carcinoma is correlated with three characteristics: size, histological architecture, and severity of dysplasia. Thus, malignant risk is highest in flat and sessile adenomas with high-grade dysplasia and a diameter > 2 cm. (5). Aside from these classic polyps or adenomas, other types can be recognized: hyperplastic polyps; mixed polyps; serrated adenomas; sessile hyperplastic polyps; inflammatory polyps and hamartomatous polyps such as juvenile polyps; Peutz-Jeghers polyps; Cowden polyps; etc. Hyperplastic polyps have a serrated epithelial architecture covered by nondysplastic epithelium. Traditionally, hyperplastic lesions have been considered as harmless, but recent evidence suggests that they can also be precursors of CRC, especially when located along the right side of the colon (6).

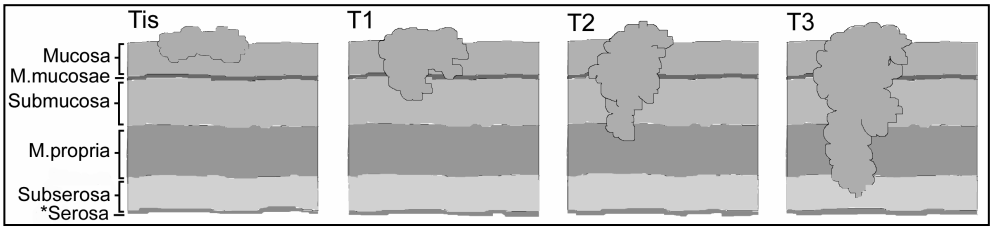

Figure 1. Pathologic staging of colorectal cancer. Staging is based on the depth of tumor invasion.* The serosa is not present in the rectum

If a tumor penetrates through the *muscularis mucosa*, the resultant carcinoma is a malignant tumor with metastatic potential. The most important prognostic indicator of colorectal carcinoma is the extent of the tumor at the time of diagnosis. Formerly, the Dukes' classification system was used; at present, the TNM classification is more standard. This system is based upon the degree of tumor penetration, lymph node metastasis, and distant metastases (Figure 1) (4). To a certain degree, this system can predict local recurrence, distant metastases, and survival rates. However, for a correct classification of colorectal cancer it is essential that the system continues to be critically evaluated and improved by evidence from clinical practice (7).

Treatment of rectal cancer: TME

For treatment of rectal cancer that invades at least the submucosa, total mesorectal excision (TME) with autonomic nerve preservation is the gold standard in The Netherlands. After a short course of preoperative radiotherapy (5*5 Gy), a low anterior resection or an abdominal peritoneal rectum amputation is performed according to the TME approach (8). Formerly, local recurrence was a major problem in rectal cancer, but with the introduction of this standardized treatment recurrence rates have declined to 2.4% two years after treatment (8). A drawback of TME surgery and radiotherapy is the associated functional morbidity, including sexual dysfunction, urologic dysfunction, and permanent colonostomy (9). Radiotherapy is only beneficial to reduce local recurrence and should therefore only be administrated to patients with high risk of local recurrence. Patients with superficial rectal cancer (T1 or T2) might be cured by surgery alone. However, current pre-treatment modalities are incapable of accurately identifying patients at high risk for local recurrence.

Treatment of rectal adenoma and early rectal cancer: TEM

Several treatment options are available for rectal adenomas. Small pedunculated adenomas can be removed by snare excision, while large sessile adenomas can be cured by transanal endoscopic microsurgery (TEM) (10). The TEM technique is an innovative technique for the local resection of rectal adenomas and has a minimal mortality and morbidity rate (10-12) (Figure 2). However, an invasive carcinoma (beyond the muscularis mucosae) is found after local excision in a large proportion of presumed benign tumors. After TEM for carcinomas, recurrence rates range from 0-14% for T1 carcinomas and 0-50% to 14-67% for T2 and T3 carcinomas, respectively (13). Since TEM involves superficial removal of the tumor, it is not possible to assess lymph node status. Hence, only those lesions with a low probability of nodal involvement should be selected for TEM treatment. The risk for lymph node metastasis varied from 10% for T1 carcinomas to 40% for T2 carcinomas (13). After an unexpected carcinoma, the patient may opt for a "wait and see" procedure with regular follow-up.

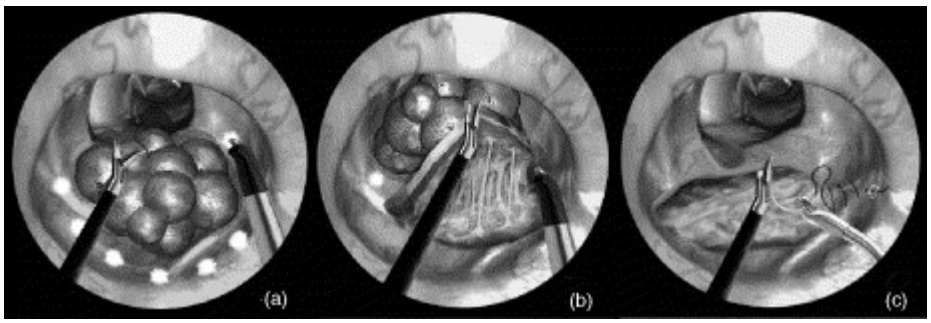

Figure 2. Illustrations of endoscopic view during TEM. The margin of excision is marked (a), after which the tumor is resected (b). The defect is closed transversally using a running suture (c). Adapted from de Graaf *et al.* (11), with permission.

However, in the case of recurrence after TEM, immediate radical TME surgery should follow, and the patient must be treated a second time. Although this has not yet been proven, T1 rectal carcinomas may be good candidates for TEM without compromising oncological outcome. For safe treatment of T1 carcinomas by TEM, low-risk criteria were established: well or moderately differentiated, no blood or lymphatic-vessel invasion, and no mucinous component. Nevertheless, the risk of lymph node metastasis is still 7% in these low-risk T1 carcinomas (11).

Preoperative staging/imaging

To make the optimal treatment choice for a rectal tumor, correct preoperative staging is essential. Previously, in presumably benign large rectal lesions, definite histopathology after operation revealed carcinomas (T1 or more) in up to 34% of tumors (14, 15), while preoperative biopsy findings were negative for carcinoma.

Modern imaging techniques have greatly improved the preoperative staging of rectal tumors, with endorectal ultrasound (ERUS), computed tomography (CT), and magnetic resonance imaging (MRI) as the most commonly used. For more advanced tumors, CT or MRI is able to assess accurately tumor invasion in other pelvic structures. ERUS seems the most suitable to assess the T stage accurately in superficial lesions (16, 17). ERUS proved able to discriminate rectal adenomas from invasive carcinomas and T1 from T2 stages. However, ERUS is not feasible in all rectal tumors and a serious problem of ERUS is over-staging (18).

Correct preoperative assessment of nodal status is still problematic. MRI with the use of ultrasmall superparamagnetic iron oxide (USPIO) contrast agents has shown promising results for staging lymph node metastasis (19). Up to now, only small studies involving

rectal cancer have been performed and the results need to be confirmed in other studies (20).

Colorectal cancer biology

Progression model for colorectal carcinogenesis

The progressive histological steps in colorectal carcinogenesis are accompanied by specific genetic changes. These are summarized in the genetic model for colorectal tumorigenesis by Fearon and Vogelstein (21) (Figure 3). In this model, normal epithelial tissue progresses via an adenoma to a carcinoma accompanied by mutations in *APC, KRAS2, SMAD4 (DPC), P53*, epigenetic changes (methylation), and genetic instability. Since the model's inception in 1990, more molecules have been added, including mismatch repair genes, *PI3K*, receptor tyrosine kinases, *PTEN, TGF-β-RII,* and E-cadherin (reviewed in (22)).

The first steps in colorectal tumorigenesis are loss of function mutations and loss of heterozygosity (LOH) at the *APC* tumor suppressor gene, which are observed in over 80% of colorectal tumors (23, 24). *APC*'s main tumor suppressor activity lies in regulating intracellular levels of β-catenin, a key member of the Wnt signal transduction pathway (25, 26). When *APC* is mutated, β-catenin accumulates and translocates to the nucleus where it modulates transcription of a broad spectrum of downstream target genes via Tcf/Lef transcription factors. The crucial role of *APC* in the colon is illustrated by inactivating mutations in *APC* that cause familial adenomatous polyposis coli (FAP), a disease characterized by hundreds to thousands of adenomas appearing in the second or third decade of life (27).

Another early event in colorectal tumorigenesis is activation of the *KRAS2* proto-oncogene. *KRAS2*-activating mutations (mainly in codons 12 and 13) are found in 50% of large adenomas and carcinomas (28, 29). *KRAS2* plays a role in the RAS-RAF-MAPK signaling pathway, which is essential for cell proliferation and differentiation (30).

Allelic deletions of chromosome 17p and 18q occur later in tumorigenesis and mark the transition from a benign adenoma to a malignant carcinoma. Originally, the *DCC* gene was indicated as the target gene of 18q loss, but more recently the *SMAD2* and *SMAD4* genes were identified for their tumor suppressor role (31, 32). The SMAD genes are

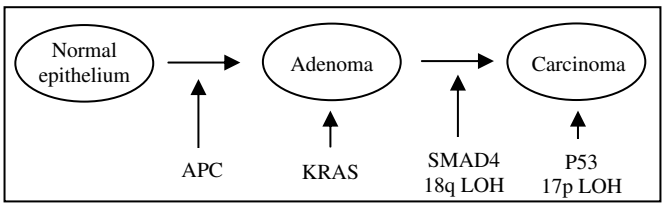

Figure 3. Adenoma-carcinoma sequence.

components of the TGF-β pathway, and inactivation affects TGF-β pathway functions like angiogenesis, cell proliferation, and differentiation (33). Most carcinomas are also characterized by the loss of p53, the tumor suppressor gene on 17p. The *p53* protein is considered the "guardian of the genome" because of its capacity to monitor the integrity of DNA (34).

Genetic instability

In addition to specific molecular changes, genetic instability is an essential requirement for cancer formation, as this leads to the higher mutation rates necessary for tumor initiation and progression. Two forms of genetic instability have been described in colorectal cancers: 1) instability at the chromosomal level (CIN); and 2) instability at the nucleotide sequence level (microsatellite instability (MSI)) (35).

CIN is characterized by gross chromosomal segregation abnormalities and is commonly detected as aneuploidy. Physical loss or gain of genetic material at specific chromosomal regions and loss of heterozygosity (LOH) both result in altered allele ratios. In addition to LOH through physical loss, copy number neutral LOH is also frequently observed at tumor suppressor loci. In this case, the wild-type allele is usually lost, whereas the mutated allele is duplicated, a process called homologous recombination. Genes involved in CIN are the *p53* tumor suppressor gene, mitotic checkpoint genes (e.g. *Bub1*), DNA damage checkpoints genes (*ATM*), and others (36). A role for *APC* in chromosomal instability was also suggested (37). The majority of colorectal cancers (75-85%) are CIN tumors and show aneuploidy; rectal cancer, especially, exhibits this main characteristic (38).

MSI tumors are characterized by the inactivation of the DNA mismatch repair (MMR) system, which causes mutation rates 2-3 fold higher than in MMR-proficient cells (2). This can be observed at short repeated sequences (microsatellites) scattered throughout the genome. Germline mutations in the same DNA MMR genes (*MLH1, MSH2, MSH6, PMS2*) are responsible for hereditary non-polyposis colorectal cancer (HNPCC or Lynch syndrome) (39). Accordingly, the majority of HNPCC tumors exhibit MSI (40). MSI also occurs in 10-15% of sporadic colorectal tumors, mainly in right-sided colon tumors by somatic inactivation of *MLH1* through promoter methylation (41).

CIN and MSI appear to be alternative pathways and generally do not occur together (42). In most colorectal cancers, one type of genetic instability is involved, but not the other. However, some studies showed subsets of MSI-stable carcinomas with diploid DNA content and no LOH. Those tumors were early-onset cases and were mostly located in the distal colon (43).

Methylation

The methylation status of DNA is important for gene expression and gene activity. In cancer, two aberrant methylation states are described: global hypomethylation and region-specific hypermethylation. Examination of DNA from adenomas revealed that approximately one-third of the DNA regions studied had lost methyl groups (44, 45). This global hypomethylation was suggested to contribute to chromosomal instability in cancers (46).

On the other hand, hypermethylation of CpG islands in promoter regions has been described (47). This leads to transcriptional silencing of tumor suppressor genes (48, 49). Hypermethylation has been increasingly associated with sporadic colorectal cancer exhibiting MSI, especially at the *MLH1* gene locus (49).

Differences between colon and rectum cancer

Various studies indicate differences in etiology, pathological features, and genetic abnormalities between colon and rectum cancer, or right-sided and left-sided CRC (50-54). Right-sided tumors are more often mucinous and diploid, and exhibit MSI and hypermethylation. Left-sided or rectal cancers have a higher frequency of CIN, 17p and 18q allelic loss, and *p53* mutations. A literature review reveals conflicting evidence concerning the prognostic significance of genes commonly implicated in the pathogenesis of colorectal carcinoma (55). One cause is the heterogeneity of study populations, in terms of both disease stage and tumor location. Although this limitation has been recognized in multiple studies, present-day sample collections are often heterogeneous.

Array profiling for classification of rectal tumors

Expression microarrays are a valuable tool for high-throughput analysis of the expression of thousands of genes in a single experiment. Genome-wide profiling has the potential for the classification and staging of cancers, a better understanding of the underlying biology, and the tracking of therapeutic improvement. A plethora of microarray studies have been performed to classify different tumor types including colorectal malignancies (56-59). One of the first successes in gene expression profiling for prognostic purposes was a study by Van‘t Veer et al. that established a 70-gene prognostic signature-predicting outcome in lymph node-negative breast cancer (60). This signature was later validated in independent studies (61, 62); the first clinical tests based on this research are now becoming available (63, 64).

Gene expression profiling of colorectal cancer

The first gene expression profiling study for colorectal cancer showed that gene expression patterns can discriminate between cancerous and normal tissues (56). Since then, gene expression signatures have been published for colorectal cancers to discriminate adenomas from carcinomas (65, 66), colon from rectum samples (67-69), and lymph node-positive cases from lymph node-negative cases (67, 70). Table 1 provides a selection of profiling studies related to tumor stage and survival. Most studies compare carcinomas with normal tissues and usually encompass a mixture of colon and rectum samples.

In the gene expression studies performed so far, the genes identified belong to a variety of pathways, including proliferation, cell adhesion, transcription, cell signalling, and many others. However, the overlap between studies is relatively small. Two recent reviews summarized all of the colorectal cancer microarray studies and concluded that despite the abundance of data, there is little overlap among the gene lists associated with specific clinical or biological phenotypes (71, 72). Tumor heterogeneity, limited cohort sizes, and methodological differences in experimental and bioinformatic approaches pose severe limitations to the comparison of different studies. So far, none of the identified classifiers for CRC have been validated in independent series or have led to a clinical application.

Genomic profiling

In contrast to gene expression studies, genomic profiling studies of colorectal cancer show more consistent results. To date, conventional CGH and array CGH studies describe specific genomic alterations related to various stages of colorectal cancer (73-76). A comprehensive meta-analysis of these studies, comprising a total of 859 cancers, allowed for the assignment of specific gain and loss events to specific tumor progression and Dukes' stages (77). In general, losses of chromosomes 17p and 18, and gains of 8q, 13q, and 20, occur at early stages in the transition from adenoma to carcinoma, whereas losses of 4p and 8p, and gains of 7p and 17q, are associated with the transition from primary tumor to metastases. Late events also include the loss of 14q and gains of 1q, 11, 12p, and 19 (77).

In addition, other studies have established chromosomal instability patterns in adenomas, or related specific aberrations to adenoma carcinoma progression. Several studies found a high incidence of chromosomal aberrations in adenomas (73-76). Hermsen *et al.* found that adenomas that have progressed to carcinomas already show many carcinoma-related chromosomal aberrations in the adenoma fractions. Leslie *et al.* found that the chromosomal loss of 17p and 18q, and the gain of 20 were related to high-grade dysplasia.

Integrative studies

Three studies integrate gene expression profiles and genomic alterations in CRC (78-80). Tsafrir *et al.* showed a good correlation between both data types and suggested a direct effect of copy number changes on gene expression (80). Particular chromosomal regions are frequently gained and over-expressed (e.g., 7p, 8q, 13q, and 20q) or lost and under-expressed (e.g., 1p, 4, 5q, 8p, 14q, 15q, and 18) in primary colon tumors. Furthermore, these aberrations are absent in normal colon mucosa, appear in benign adenomas (albeit only in a small fraction of samples), become more frequent as the disease advances, and are found in the majority of metastatic samples. Similarly, Habermann *et al.* (79) correlated gene expression, genomic profiles, and proteomic profiles with different tumor stages.

SNP arrays for copy number and LOH analysis

Single nucleotide polymorphism (SNP) arrays allow high-resolution genome-wide genotyping for various applications, including linkage and association studies (81, 82). In addition to genotyping, these arrays can also be used to detect genomic abnormalities, such as copy number changes and LOH, the latter of which cannot be detected by (array) CGH analysis (83, 84). Beroukhim *et al.* used SNP arrays in different tumor types, revealing that 80% of all LOH events are caused by copy-neutral or copy-gain events (85). For colorectal cancer, Andersen *et al*. showed that half of the identified LOH regions had no evidence of reduced copy number (86). The distribution of these structures was non-random, and primarily involved 8q, 13q, and 20q. However, a major obstacle in the use of SNP arrays is the requirement of most platforms for high-quality DNA from freshly frozen tissue or leukocytes; often, these are not available for large retrospective tumor series.

Table 1. List of published studies showing expression profiling data for CRC progression stages and recurrence.

Study	Comparison	Location	Sample size	Platform	Identified genes and Conclusions
Normal vs. Adenoma					
Lin et al. (65)	normal vs. adenoma adenoma vs. carcinoma	colon	9 adenomas, 11 carcinomas	cDNA	51 genes up-regulated, 376 down-regulated in both types of tumors vs.normal 50 genes significantly different between adenomas and carcinomas
Notterman et al. (66)	normal vs. adenoma normal vs. carcinoma	colon	18 carc, 4 ad	Affymetrix	19 transcripts higher, 47 lower compared to normal Hierarchical clustering separated adenoma from carcinoma and normal
Normal vs. Carcinoma (different stages)					
Agrawal et al. (87)	normal vs. carcinoma, metastatic vs. non-metastatic carcinoma (liver metastasis)	unspecified	60 samples (ad, car, liver met) samples pooled	Affymetrix	300 tumor markers, 100 progression markers Osteopontin identified as progression marker
Alon et al. (56)	normal vs. carcinoma	colon	40 tumors	Affymetrix	Two way clustering revealed co-regulated families of genes (i.e., ribosomal proteins) Clustering separated cancerous from non-cancerous and cell lines from in vivo tissues
Birkenkamp-Demtroder el al. (68)	normal vs. carcinoma right-vs. left sided CRC	Caecum, rectosigmoid, sigmoid	45 dukes B and C	Affymetrix	58 genes relating to specific colonic location (i.e.pS2, S100P, sialyltransferase) A total of 118 and 186 genes were different between normal and tumor 30 genes different between normal and tumor, including matrix metalloproteinases. Side specific differences in tumor expression: keratins, carbonic anhydrases, and COX2
Groene et al. (88)	UICC II vs. III	colon and rectum	36 UICC II and III	Affymetrix	45 probe sets different between UICC stage II and III . The most distinctive elements were GSPT2 and HOXA9. No substantial differential expression of genes in cancer-related pathways
Takemasa et al. (89)	normal vs. carcinoma	unspecified	16 samples	cDNA	Colonochip generated containing 4608 cDNA clones and 170 genes 59 genes showed twofold differential expression between cancer and normal mucosa.
Lymph node metastasis					
Bertucci et al. (67)	normal vs. carcinoma metastatic vs. non-metastatic carcinoma lymph node positive vs. negative MSI vs. MSS carcinoma left vs. right carcinoma	colon and rectum	22 samples	cDNA nylon membrame	Clustering was able to distinguish normal vs. cancer tissues and metastatic vs. nonmetastatic tumors Supervised analyses segregated tumors on basis of histology, LN metastastasis, genetic instability, location, and different 5-year survival NM23 was validated on a tissue array, showing down regulation in poor prognosis
Koehler et al. (70)	normal vs. carcinoma, lymph node positive vs. negative (low stage vs. high stage (T4 and LN))	colon and rectum	24 carcinomas 14 liver metastasis	cDNA nylon membrame	40 genes related to malignancy, 23 to high stage (T4, LN) The 23 gene set may represent important targets in colorectal carcinogenesis and might provide useful clinicopathological tools
Recurrence					
Arango et al. (90)	recurrence in Dukes' C	unspecified	25 samples	Affymetrix	Clustering reveals several groups of patients, RHOA was validated by tissue microarray and associated with survival gene expression profiling can predict recurrence in Dukes'C patients.
Barrier et al. (91)	recurrence in Dukes' B and C	right and left sided colon cancer	18 samples	Affymetrix	30 gene set based on tumor tissue, 70 gene set based on normal mucosa Accurate prognosis predictor can be build on gene expression measurements
Wang et al. (92)	recurrence in Dukes' B	colon	74 samples	Affymetrix	23-gene signature predict recurrence, accuracy was 78%, validated in 36 patients Our data highlight the feasiblity of a prognostic assay that could focus more intensive treatment for localized colon cancer.

Aims of the study and outline of this thesis

Aim of the study

Accurate staging of rectal tumors is essential for choosing the correct treatment. Therefore, the identification of preoperative parameters to correctly assess aggressive tumor behavior is essential.

The aim of this thesis is to identify molecular differences between rectal tumors of different stages using genomic analysis and gene expression profiling. Ultimately, these differences should lead to a clear distinction between benign adenomas and adenomas containing a small invasive focus, and between carcinomas without lymph node metastasis and those with lymph node metastasis. Those molecular differences could hopefully be applied as markers for better preoperative diagnosis of rectal tumors. For such a clinical application, it is important that the methodology is applicable to formalin-fixed, paraffin-embedded (FFPE) material: the limited amount of material obtained in biopsies can then be used for molecular analysis as well as standard histopathology and immunohistochemistry.

Outline of this thesis

Chapters 2 and 3 are dedicated to the development of protocols and algorithms for a newly developed SNP array platform. In Chapter 2, genotyping and LOH analysis of FFPE tissue is tested with these SNP arrays and data from FFPE and frozen tumor samples are compared. In Chapter 3, new software algorithms for the Illumina SNP array platform for copy number analysis are developed and applied to a well-characterized set of colorectal tumors.

In Chapter 4, frozen tissue and FFPE fractions of TEM and TME-treated rectal samples of various tumor stages are analyzed for LOH and copy number changes using SNP arrays. Furthermore, adenoma and carcinoma fractions of single cases are compared. In Chapter 5, the latter series is extended. This series consists of early carcinomas treated by TEM which are not recognized preoperatively as carcinomas. In addition, intra-tumor heterogeneity is assessed in three different *ex vivo* biopsies per patient. In Chapter 6, gene expression array studies are performed on the same samples as the profiling genomic study (Chapter 4) and the chromosomal instability patterns are integrated with the gene expression array data. Data of the expression array experiments are validated by other methods. Finally, Chapter 7 contains concluding remarks and implications for further research.

References

1. Midgley R, Kerr D: Colorectal cancer. Lancet 353:391-399, 1999
2. Kinzler KW, Vogelstein B: Lessons from hereditary colorectal cancer. Cell 87:159-170, 1996
3. Jemal A, Thomas A, Murray T, et al.: Cancer statistics, 2002. CA Cancer J Clin 52:23-47, 2002
4. Kumar V, Abbas AK, Fausto N: Pathologic basis of disease, 2005
5. Wolber RA, Owen DA: Flat adenomas of the colon. Hum Pathol 22:70-74, 1991
6. Jass JR: Hyperplastic polyps and colorectal cancer: is there a link? Clin Gastroenterol Hepatol 2:1-8, 2004
7. Quirke P, Williams GT, Ectors N, et al.: The future of the TNM staging system in colorectal cancer: time for a debate? Lancet Oncol 8:651-657, 2007
8. Kapiteijn E, Marijnen CA, Nagtegaal ID, et al.: Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 345:638-646, 2001
9. Peeters KC, van de Velde CJ, Leer JW, et al.: Late side effects of short-course preoperative radiotherapy combined with total mesorectal excision for rectal cancer: increased bowel dysfunction in irradiated patients--a Dutch colorectal cancer group study. J Clin Oncol 23:6199-6206, 2005
10. Buess G, Mentges B, Manncke K, et al.: Technique and results of transanal endoscopic microsurgery in early rectal cancer. Am J Surg 163:63-69, 1992
11. de Graaf EJ, Doornebosch PG, Stassen LP, et al.: Transanal endoscopic microsurgery for rectal cancer. Eur J Cancer 38:904-910, 2002
12. Rokke O, Iversen KB, Ovrebo K, et al.: Local resection of rectal tumors by transanal endoscopic microsurgery: experience with the first 70 cases. Dig Surg 22:182-189, 2005
13. Maslekar S, Beral DL, White TJ, et al.: Transanal endoscopic microsurgery: where are we now? Dig Surg 23:12-22, 2006
14. Galandiuk S, Fazio VW, Jagelman DG, et al.: Villous and tubulovillous adenomas of the colon and rectum. A retrospective review, 1964-1985. Am J Surg 153:41-47, 1987
15. Taylor EW, Thompson H, Oates GD, et al.: Limitations of biopsy in preoperative assessment of villous papilloma. Dis Colon Rectum 24:259-262, 1981
16. Beets-Tan RG, Beets GL: Rectal cancer: review with emphasis on MR imaging. Radiology 232:335-346, 2004
17. Bipat S, Glas AS, Slors FJ, et al.: Rectal cancer: local staging and assessment of lymph node involvement with endoluminal US, CT, and MR imaging--a meta-analysis. Radiology 232:773-783, 2004
18. Doornebosch PG, Bronkhorst PJ, Hop WC, et al.: The Role of Endorectal Ultrasound in Therapeutic Decision-Making for Local vs. Transabdominal Resection of Rectal Tumors. Dis Colon Rectum, 2007
19. Harisinghani MG, Barentsz J, Hahn PF, et al.: Noninvasive detection of clinically occult lymph-node metastases in prostate cancer. N Engl J Med 348:2491-2499, 2003
20. Koh DM, Brown G, Temple L, et al.: Rectal cancer: mesorectal lymph nodes at MR imaging with USPIO versus histopathologic findings--initial observations. Radiology 231:91-99, 2004
21. Fearon ER, Vogelstein B: A genetic model for colorectal tumorigenesis. Cell 61:759-767, 1990
22. Houlston RS: What we could do now: molecular pathology of colorectal cancer. Mol Pathol 54:206-214, 2001
23. Miyaki M, Konishi M, Kikuchi-Yanoshita R, et al.: Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. Cancer Res 54:3011-3020, 1994
24. Powell SM, Zilz N, Beazer-Barclay Y, et al.: APC mutations occur early during colorectal tumorigenesis. Nature 359:235-237, 1992
25. Fodde R, Smits R, Clevers H: APC, signal transduction and genetic instability in colorectal cancer. Nat Rev Cancer 1:55-67, 2001
26. Reya T, Clevers H: Wnt signalling in stem cells and cancer. Nature 434:843-850, 2005
27. Groden J, Thliveris A, Samowitz W, et al.: Identification and characterization of the familial adenomatous polyposis coli gene. Cell 66:589-600, 1991

28. Andreyev HJ, Norman AR, Cunningham D, et al.: Kirsten ras mutations in patients with colorectal cancer: the multicenter "RASCAL" study. J Natl Cancer Inst 90:675-684, 1998
29. Vogelstein B, Fearon ER, Hamilton SR, et al.: Genetic alterations during colorectal-tumor development. N Engl J Med 319:525-532, 1988
30. Bos JL: ras oncogenes in human cancer: a review. Cancer Res 49:4682-4689, 1989
31. Eppert K, Scherer SW, Ozcelik H, et al.: MADR2 maps to 18q21 and encodes a TGFbeta-regulated MAD-related protein that is functionally mutated in colorectal carcinoma. Cell 86:543-552, 1996
32. Thiagalingam S, Lengauer C, Leach FS, et al.: Evaluation of candidate tumour suppressor genes on chromosome 18 in colorectal cancers. Nat Genet 13:343-346, 1996
33. Shi Y, Massague J: Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell 113:685-700, 2003
34. Lane DP: Cancer. p53, guardian of the genome. Nature 358:15-16, 1992
35. Lengauer C, Kinzler KW, Vogelstein B: Genetic instability in colorectal cancers. Nature 386:623-627, 1997
36. Cahill DP, Lengauer C, Yu J, et al.: Mutations of mitotic checkpoint genes in human cancers. Nature 392:300-303, 1998
37. Kaplan KB, Burds AA, Swedlow JR, et al.: A role for the Adenomatous Polyposis Coli protein in chromosome segregation. Nat Cell Biol 3:429-432, 2001
38. Lanza G, Jr., Maestri I, Dubini A, et al.: p53 expression in colorectal cancer: relation to tumor type, DNA ploidy pattern and short-term survival. Am J Clin Pathol 105:604-612, 1996
39. Lynch HT, Smyrk T: Hereditary nonpolyposis colorectal cancer (Lynch syndrome). An updated review. Cancer 78:1149-1167, 1996
40. Aaltonen LA, Peltomaki P, Leach FS, et al.: Clues to the pathogenesis of familial colorectal cancer. Science 260:812-816, 1993
41. Cunningham JM, Christensen ER, Tester DJ, et al.: Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 58:3455-3460, 1998
42. Lengauer C, Kinzler KW, Vogelstein B: Genetic instabilities in human cancers. Nature 396:643-649, 1998
43. Chan TL, Curtis LC, Leung SY, et al.: Early-onset colorectal cancer with stable microsatellite DNA and near-diploid chromosomes. Oncogene 20:4871-4876, 2001
44. Goelz SE, Vogelstein B, Hamilton SR, et al.: Hypomethylation of DNA from benign and malignant human colon neoplasms. Science 228:187-190, 1985
45. Feinberg AP, Gehrke CW, Kuo KC, et al.: Reduced genomic 5-methylcytosine content in human colonic neoplasia. Cancer Res 48:1159-1161, 1988
46. Lengauer C, Kinzler KW, Vogelstein B: DNA methylation and genetic instability in colorectal cancer cells. Proc Natl Acad Sci U S A 94:2545-2550, 1997
47. Esteller M: Dormant hypermethylated tumour suppressor genes: questions and answers. J Pathol 205:172-180, 2005
48. Herman JG, Merlo A, Mao L, et al.: Inactivation of the CDKN2/p16/MTS1 gene is frequently associated with aberrant DNA methylation in all common human cancers. Cancer Res 55:4525-4530, 1995
49. Wheeler JM, Beck NE, Kim HC, et al.: Mechanisms of inactivation of mismatch repair genes in human colorectal cancer cell lines: the predominant role of hMLH1. Proc Natl Acad Sci U S A 96:10296-10301, 1999
50. Beart RW, Melton LJ, III, Maruta M, et al.: Trends in right and left-sided colon cancer. Dis Colon Rectum 26:393-398, 1983
51. Frattini M, Balestra D, Suardi S, et al.: Different genetic features associated with colon and rectal carcinogenesis. Clin Cancer Res 10:4015-4021, 2004
52. Kapiteijn E, Liefers GJ, Los LC, et al.: Mechanisms of oncogenesis in colon versus rectal cancer. J Pathol 195:171-178, 2001
53. Scott N, Sagar P, Stewart J, et al.: p53 in colorectal cancer: clinicopathological correlation and prognostic significance. Br J Cancer 63:317-319, 1991
54. Thibodeau SN, Bren G, Schaid D: Microsatellite instability in cancer of the proximal colon. Science 260:816-819, 1993
55. Anwar S, Frayling IM, Scott NA, et al.: Systematic review of genetic influences on the prognosis of colorectal cancer. Br J Surg 91:1275-1291, 2004

56. Alon U, Barkai N, Notterman DA, et al.: Broad patterns of gene expression revealed by clustering analysis of tumor and normal colon tissues probed by oligonucleotide arrays. Proc Natl Acad Sci U S A 96:6745-6750, 1999
57. Golub TR, Slonim DK, Tamayo P, et al.: Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science 286:531-537, 1999
58. Hedenfalk I, Duggan D, Chen Y, et al.: Gene-expression profiles in hereditary breast cancer. N Engl J Med 344:539-548, 2001
59. Perou CM, Sorlie T, Eisen MB, et al.: Molecular portraits of human breast tumours. Nature 406:747-752, 2000
60. 't Veer LJ, Dai H, Van de Vijver MJ, et al.: Gene expression profiling predicts clinical outcome of breast cancer. Nature 415:530-536, 2002
61. Buyse M, Loi S, van't Veer L, et al.: Validation and clinical utility of a 70-gene prognostic signature for women with node-negative breast cancer. J Natl Cancer Inst 98:1183-1192, 2006
62. Van de Vijver MJ, He YD, van't Veer LJ, et al.: A gene-expression signature as a predictor of survival in breast cancer. N Engl J Med 347:1999-2009, 2002
63. Garber K: Genomic medicine. Gene expression tests foretell breast cancer's future. Science 303:1754-1755, 2004
64. Kallioniemi O: Medicine: profile of a tumour. Nature 428:379-382, 2004
65. Lin YM, Furukawa Y, Tsunoda T, et al.: Molecular diagnosis of colorectal tumors by expression profiles of 50 genes expressed differentially in adenomas and carcinomas. Oncogene 21:4120-4128, 2002
66. Notterman DA, Alon U, Sierk AJ, et al.: Transcriptional gene expression profiles of colorectal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. Cancer Res 61:3124-3130, 2001
67. Bertucci F, Salas S, Eysteries S, et al.: Gene expression profiling of colon cancer by DNA microarrays and correlation with histoclinical parameters. Oncogene 23:1377-1391, 2004
68. Birkenkamp-Demtroder K, Olesen SH, Sorensen FB, et al.: Differential gene expression in colon cancer of the caecum versus the sigmoid and rectosigmoid. Gut 54:374-384, 2005
69. Komuro K, Tada M, Tamoto E, et al.: Right- and left-sided colorectal cancers display distinct expression profiles and the anatomical stratification allows a high accuracy prediction of lymph node metastasis. J Surg Res 124:216-224, 2005
70. Koehler A, Bataille F, Schmid C, et al.: Gene expression profiling of colorectal cancer and metastases divides tumours according to their clinicopathological stage. J Pathol 204:65-74, 2004
71. Cardoso J, Boer J, Morreau H, et al.: Expression and genomic profiling of colorectal cancer. Biochim Biophys Acta, 2006
72. Shih W, Chetty R, Tsao MS: Expression profiling by microarrays in colorectal cancer (Review). Oncol Rep 13:517-524, 2005
73. Hermsen M, Postma C, Baak J, et al.: Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. Gastroenterology 123:1109-1119, 2002
74. Hoglund M, Gisselsson D, Hansen GB, et al.: Dissecting karyotypic patterns in colorectal tumors: two distinct but overlapping pathways in the adenoma-carcinoma transition. Cancer Res 62:5939-5946, 2002
75. Leslie A, Stewart A, Baty DU, et al.: Chromosomal changes in colorectal adenomas: relationship to gene mutations and potential for clinical utility. Genes Chromosomes Cancer 45:126-135, 2006
76. Ried T, Knutzen R, Steinbeck R, et al.: Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosomes Cancer 15:234-245, 1996
77. Diep CB, Kleivi K, Ribeiro FR, et al.: The order of genetic events associated with colorectal cancer progression inferred from meta-analysis of copy number changes. Genes Chromosomes Cancer 45:31-41, 2006
78. Grade M, Ghadimi BM, Varma S, et al.: Aneuploidy-dependent massive deregulation of the cellular transcriptome and apparent divergence of the Wnt/beta-catenin signaling pathway in human rectal carcinomas. Cancer Res 66:267-282, 2006

79. Habermann JK, Paulsen U, Roblick UJ, et al.: Stage-specific alterations of the genome, transcriptome, and proteome during colorectal carcinogenesis. Genes Chromosomes Cancer 46:10-26, 2007
80. Tsafrir D, Bacolod M, Selvanayagam Z, et al.: Relationship of gene expression and chromosomal abnormalities in colorectal cancer. Cancer Res 66:2129-2137, 2006
81. Fan JB, Chee MS, Gunderson KL: Highly parallel genomic assays. Nat Rev Genet 7:632-644, 2006
82. Matsuzaki H, Loi H, Dong S, et al.: Parallel genotyping of over 10,000 SNPs using a one-primer assay on a high-density oligonucleotide array. Genome Res 14:414-425, 2004
83. Bignell GR, Huang J, Greshock J, et al.: High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res 14:287-295, 2004
84. Zhao X, Li C, Paez JG, et al.: An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. Cancer Res 64:3060-3071, 2004
85. Beroukhim R, Lin M, Park Y, et al.: Inferring loss-of-heterozygosity from unpaired tumors using high-density oligonucleotide SNP arrays. PLoS Comput Biol 2:e41, 2006
86. Andersen CL, Wiuf C, Kruhoffer M, et al.: Frequent occurrence of uniparental disomy in colorectal cancer. Carcinogenesis 28:38-48, 2007
87. Agrawal D, Chen T, Irby R, et al.: Osteopontin identified as lead marker of colon cancer progression, using pooled sample expression profiling. J Natl Cancer Inst 94:513-521, 2002
88. Groene J, Mansmann U, Meister R, et al.: Transcriptional census of 36 microdissected colorectal cancers yields a gene signature to distinguish UICC II and III. Int J Cancer 119:1829-1836, 2006
89. Takemasa I, Higuchi H, Yamamoto H, et al.: Construction of preferential cDNA microarray specialized for human colorectal carcinoma: molecular sketch of colorectal cancer. Biochem Biophys Res Commun 285:1244-1249, 2001
90. Arango D, Laiho P, Kokko A, et al.: Gene-expression profiling predicts recurrence in dukes' C colorectal cancer. Gastroenterology 129:874-884, 2005
91. Barrier A, Lemoine A, Boelle PY, et al.: Colon cancer prognosis prediction by gene expression profiling. Oncogene 24:6155-6164, 2005
92. Wang Y, Jatkoe T, Zhang Y, et al.: Gene expression profiles and molecular markers to predict recurrence of Dukes' B colon cancer. J Clin Oncol 22:1564-1571, 2004