

Gain of Chromosome 8q23–24 Is a Predictive Marker for Lymph Node Positivity in Colorectal Cancer

B. Michael Ghadimi,¹ Marian Grade,²
Torsten Liersch, Claus Langer, Alexander Siemer,
László Füzesi, and Heinz Becker

Department of General, Visceral, and Transplantation Surgery [B. M. G., M. G., T. L., C. L., H. B.], Institute of Pathology [L. F.], and Department of Medical Statistics [A. S.], University Medical Center Göttingen, 37075 Göttingen, Germany

ABSTRACT

Purpose: The prognosis of patients with colorectal cancer is largely determined by tumor stage. In this respect, colorectal cancers with lymph node metastases indicate a worse prognosis versus lymph node-negative tumors. Accordingly, there is considerable clinical interest in understanding the genetic mechanisms underlying metastasis formation. Furthermore, sensitive and specific biomarkers are needed to predict the metastatic phenotype at the time of diagnosis.

Experimental Design: Fifty colorectal cancers with or without lymph node metastases were assessed for genomic imbalances by comparative genomic hybridization. Particular interest was focused on whether specific chromosomal alterations exist in primary tumors that might be indicative and specific for the metastatic phenotype.

Results: The analysis revealed that lymph node-positive colorectal cancers show a higher degree of chromosomal instability than lymph node-negative cancers (average number of chromosomal copy alterations, 9.8 versus 7.5). Chromosomal alterations commonly described in colorectal cancers such as gain of 20q or loss of 18q21 were not different. However, the gain of chromosomal region 8q23–24 was seen in the vast majority of lymph node-positive cancers, whereas it was rather rare in lymph node-negative carcinomas ($P = 0.0016$).

Conclusions: These data suggest that genes located at 8q23–24 might favor the development of lymphatic metastases in colorectal cancers. Additionally, the gain of this region could be used to predict the metastatic potential of primary colorectal cancers.

INTRODUCTION

The prognosis of patients with colorectal cancer is largely determined by the tumor stage UICC.³ In this respect, both lymph node and hepatic metastases indicate advanced disease with an unfavorable prognosis. At the time of diagnosis, approximately 60% of colorectal cancers have already formed lymph node metastases, and in this respect, rectal cancers might benefit from neoadjuvant radiochemotherapy before surgical resection (1). There is thus considerable clinical interest in understanding the genetic mechanisms underlying metastasis formation. Furthermore, sensitive and specific biomarkers are needed to predict the metastatic phenotype at the time of diagnosis. In recent years, gene-by-gene analysis has not fully succeeded in coming up with a comprehensive understanding of the mechanisms that enable individual cancers to form lymph node metastases (2). In the field of colorectal cancer, several studies have analyzed the progression of colorectal adenomas to invasive cancers and found a stage-specific chromosomal aberration pattern indicating the sequential emergence of chromosomal gains and losses (3, 4). These nonrandom, tumor type-specific chromosomal alterations include gains of chromosome 1, 7p, 8q, 13, and 20. Chromosomal losses frequently map to chromosome 4, 8p, 10q, 17p, and 18q (3, 5). However, there is considerable scientific and clinical interest in locating tumor stage-dependent chromosomal regions to find genes that may be responsible for tumor progression and using such hot spots as predictive biomarkers for a pretherapeutic molecular staging and individual risk estimation. In this respect, considerable work has been done to study the changes that occur between primary colorectal cancer and its hepatic metastases. It has been found that gains of chromosome 6q, 7q, 8q, 13q, and 20q occur frequently in hepatic metastases and might pinpoint relevant genomic loci that are necessary for the formation of metastases (6–9). However, no detailed study has been published assessing the chromosomal profiles of colorectal cancers with the capacity for local lymphatic spread, which represent the majority of cancers.

The purpose of this study was to investigate the genomic differences in colorectal cancer progression with respect to the extent of tumor infiltration into the colonic wall and, in particular, the capacity of the individual tumor to form lymphatic metastases. We therefore screened three groups of colorectal cancers with CGH to search for chromosomal alterations that might be responsible for local tumor growth and to delineate genomic regions that might indicate the lymphatic metastatic phenotype.

Received 7/9/02; revised 11/25/02; accepted 11/25/02.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹ To whom requests for reprints should be addressed, at Department of General Surgery, Georg-August-University Göttingen, Robert-Koch-Strasse 40, D-37075 Göttingen, Germany. E-mail: mghadimi@surgery-goettingen.de.

² This manuscript is part of the doctoral thesis of M.G.

³ The abbreviations used are: UICC, International Union Against Cancer; CGH, comparative genomic hybridization; ANCA, average number of chromosomal copy alterations.

Table 1 Clinicopathological data of 50 patients with colorectal cancer

	Age (yrs)	Sex	Histology	Localization
Group 1 (T ₂ , N ₀)				
1	79	M	pT ₂ , pN ₀	Sigma
2	80	F	pT ₂ , pN ₀	Colon
3	73	M	pT ₂ , pN ₀	Colon
4	88	F	pT ₂ , pN ₀	Rectum
5	69	M	pT ₂ , pN ₀	Rectum
6	79	M	pT ₂ , pN ₀	Rectum
7	61	F	pT ₂ , pN ₀	Rectum
8	88	F	pT ₂ , pN ₀	Rectum
9	63	F	pT ₂ , pN ₀	Colon
10	57	M	pT ₂ , ypN ₀	Rectum
11	68	M	pT ₂ , pN ₀	Colon
12	67	M	pT ₂ , pN ₀	Rectum
13	81	M	pT ₂ , pN ₀	Rectum
14	65	F	pT ₂ , pN ₀	Rectum
15	82	M	pT ₂ , pN ₀	Colon
Group 2 (T ₃₋₄ , N ₀)				
16	54	M	pT ₃ , pN ₀	Colon
17	60	F	pT ₃ , pN ₀	Rectum
18	53	M	pT ₃ , pN ₀	Colon
19	67	M	pT ₃ , pN ₀	Colon
20	72	F	pT ₃ , pN ₀	Colon
21	73	M	pT ₃ , pN ₀	Colon
22	63	M	pT ₃ , pN ₀	Colon
23	64	M	pT ₃ , pN ₀	Rectum
24	66	M	pT ₃ , pN ₀	Rectum
25	68	F	pT ₃ , pN ₀	Colon
26	74	F	pT ₃ , pN ₀	Rectum
27	55	M	pT ₄ , pN ₀	Colon
28	29	M	pT ₄ , pN ₀	Colon
29	76	F	pT ₄ , pN ₀	Colon
30	75	M	pT ₄ , pN ₀	Colon
Group 3 (T ₃₋₄ , N ₁₋₃)				
31	52	M	pT ₃ , pN ₁	Colon
32	54	F	pT ₃ , pN ₁	Colon
33	73	M	pT ₃ , pN ₁	Colon
34	67	M	pT ₃ , pN ₁	Colon
35	66	M	pT ₃ , pN ₁	Colon
36	83	F	pT ₄ , pN ₁	Colon
37	54	M	pT ₃ , pN ₂	Rectum
38	71	M	pT ₃ , pN ₂	Colon
39	76	M	pT ₃ , pN ₂	Colon
40	60	M	pT ₃ , pN ₂	Rectum
41	66	F	pT ₃ , pN ₂	Colon
42	66	M	pT ₃ , pN ₂	Colon
43	68	F	pT ₃ , pN ₂	Rectum
44	65	F	pT ₃ , pN ₂	Colon
45	42	M	pT ₃ , pN ₂	Colon
46	89	F	pT ₃ , pN ₂	Rectum
47	65	M	pT ₄ , pN ₂	Rectum
48	69	F	pT ₄ , pN ₂	Colon
49	85	M	pT ₄ , pN ₂	Colon
50	77	M	pT ₄ , pN ₂	Colon

PATIENTS AND METHODS

Patient Material. In the present study, surgical specimens from 50 patients diagnosed with a colorectal cancer between 1998 and 2001 were analyzed. Only fresh frozen tumor samples with a tumor cell content of at least 70% were studied. The histopathological classification was based on the WHO histological typing of colorectal cancers (UICC, 1997). All tumors were adenocarcinomas. The clinical data are summarized in Table 1. Three groups of tumors were analyzed to delineate the differences between small nonmetastasizing tumors (group 1, T₂, N₀; n = 15) versus large nonmetastasizing

tumors (group 2, T₃₋₄, N₀; n = 15) versus large tumors metastasizing into the surrounding lymph nodes (group 3, T₃₋₄, N₁₋₂; n = 20).

CGH. CGH experiments and analysis were performed as described previously (10). Briefly, CGH was performed on normal, sex-matched metaphase chromosomes prepared according to standard procedures following the criteria of du Manoir *et al.* (11). Control DNA was labeled with digoxigenin-12-dUTP (Boehringer Mannheim, Mannheim, Germany) by nick translation. Tumor DNA was extracted from colorectal cancers using a commercially available DNA isolation kit from Qiagen (Hilden, Germany). Labeling of genomic tumor DNA was performed by nick translation, substituting dTTP with biotin-16-dUTP (Boehringer Mannheim). Three hundred ng of each differentially labeled genome were precipitated together with an excess (30 μg) of the Cot-1 fraction of human DNA (Life Technologies, Inc., Gaithersburg, MD). The probe DNA was resuspended in 10 μl of hybridization solution (50% formamide, 2× SSC, and 10% dextran sulfate), denatured (5 min, 75°C), and preannealed for 1 h at 37°C. The normal metaphase chromosomes were denatured separately (70% formamide and 2× SSC) for 2 min at 75°C. Hybridization took place under a coverslip for 2 days at 37°C. Posthybridization washes and immunocytochemical detection were performed as described previously (10). Biotin-labeled tumor sequences were detected with avidin conjugated to FITC (Vector laboratories), and the digoxigenin-labeled reference DNA was developed using a mouse antidigoxin antibody, followed by a TRITC-conjugated antimouse antibody (Sigma, St. Louis, MO). The slides were counterstained with 4',6-diamidino-2-phenylindole and embedded in an antifade solution containing para-phenylenediamine (Sigma).

Gray level images were acquired for each fluorochrome using a cooled charge-coupled device camera (Sensys, Photometrics, Munich, Germany) coupled to an epifluorescence microscope (Axiovert 25; Zeiss, Jena, Germany), using sequential exposure through fluorochrome-specific filters. For automated karyotyping and analysis, a software package was used (Quips Karyotyping/CGH; Vysis). The karyograms (see Figs. 1–3) summarize the individual CGH experiments for the tumors. The *lines to the left* of the chromosomal ideograms indicate chromosomal losses (ratio, 0.75), and the *lines to the right* indicate chromosomal gains (ratio, 1.25). Amplifications are drawn as *bold lines*. Genomic instability was estimated as the ANCA/case (for details, see Ref. 12).

Statistical Analysis. The first part of the statistical analysis was done by pairwise comparisons of the three groups. All hypotheses were tested in a two-sided test at a level of 5%. Fisher's exact test was used to determine the significance values. Differences with a *P* < 0.05 were considered statistically significant.

RESULTS

CGH analysis was performed in 50 patients with colorectal cancer. These cancers could be assigned to three different groups according to the UICC classification: group 1 (pT₂, N₀),

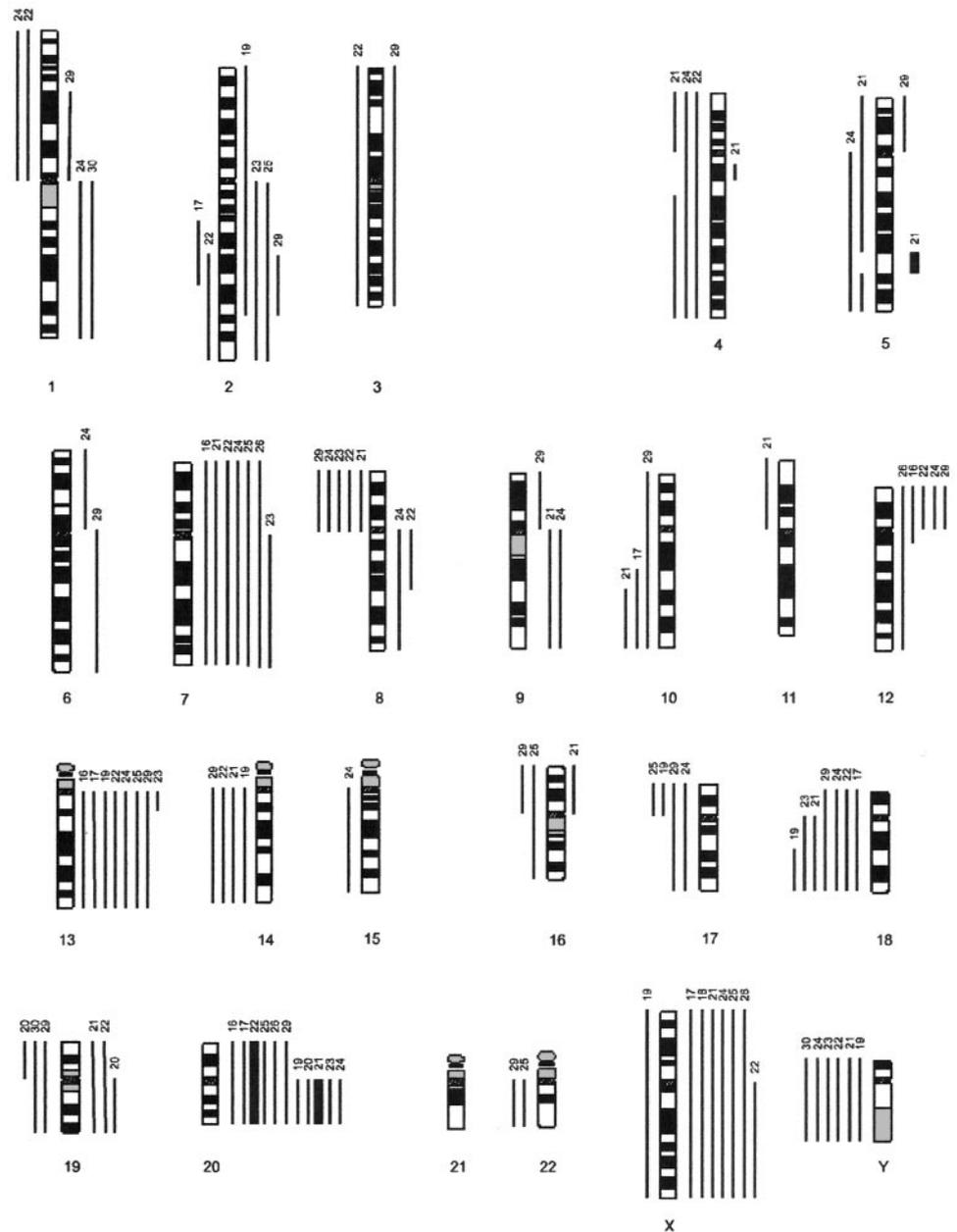


Fig. 2 Karyogram of chromosomal gains and losses in group 2 (T_{3-4} , N_0).

Detailed analysis revealed losses at 18q21-ter in 14 of 20 studied colorectal cancers (70%; Fig. 3).

Comparison of Nonmetastasizing versus Metastasizing Colorectal Cancers. In group 3, the highest amount of chromosomal instability was found reflected by an ANCA of 9.8 versus 5.7 in group 1. The frequency of amplifications was not different in the three studied groups. Also, gains of 20q and losses of 18q21, often described as markers of advanced colorectal cancer, were not statistically different between the three groups. However, a comparison of groups 1 and group 2, which differed only in the depth of infiltration into the colonic wall, revealed an increase of gains of chromosome 7p from 7% in group 1 to 45% in group 2. Although, this difference was not statistically significant ($P = 0.08$), it also prevailed in group 3.

A major finding of our study was that gains of chromosome 8q23–24 occurred in the vast majority of lymph node-positive colorectal cancers (70%) versus only 7% in lymph node-negative cancers ($P = 0.0016$; Fig. 4).

DISCUSSION

In recent years, progress has been achieved in the treatment of colorectal cancer. New therapeutic regimens have been adopted including neoadjuvant radiochemotherapies for locally advanced rectal cancers (1, 13, 14) as well as new chemotherapeutic agents for metastasizing colon cancers (15). Nonetheless, the metastatic potential of tumors, especially to regional lymph nodes, is the major obstacle to successful treatment for

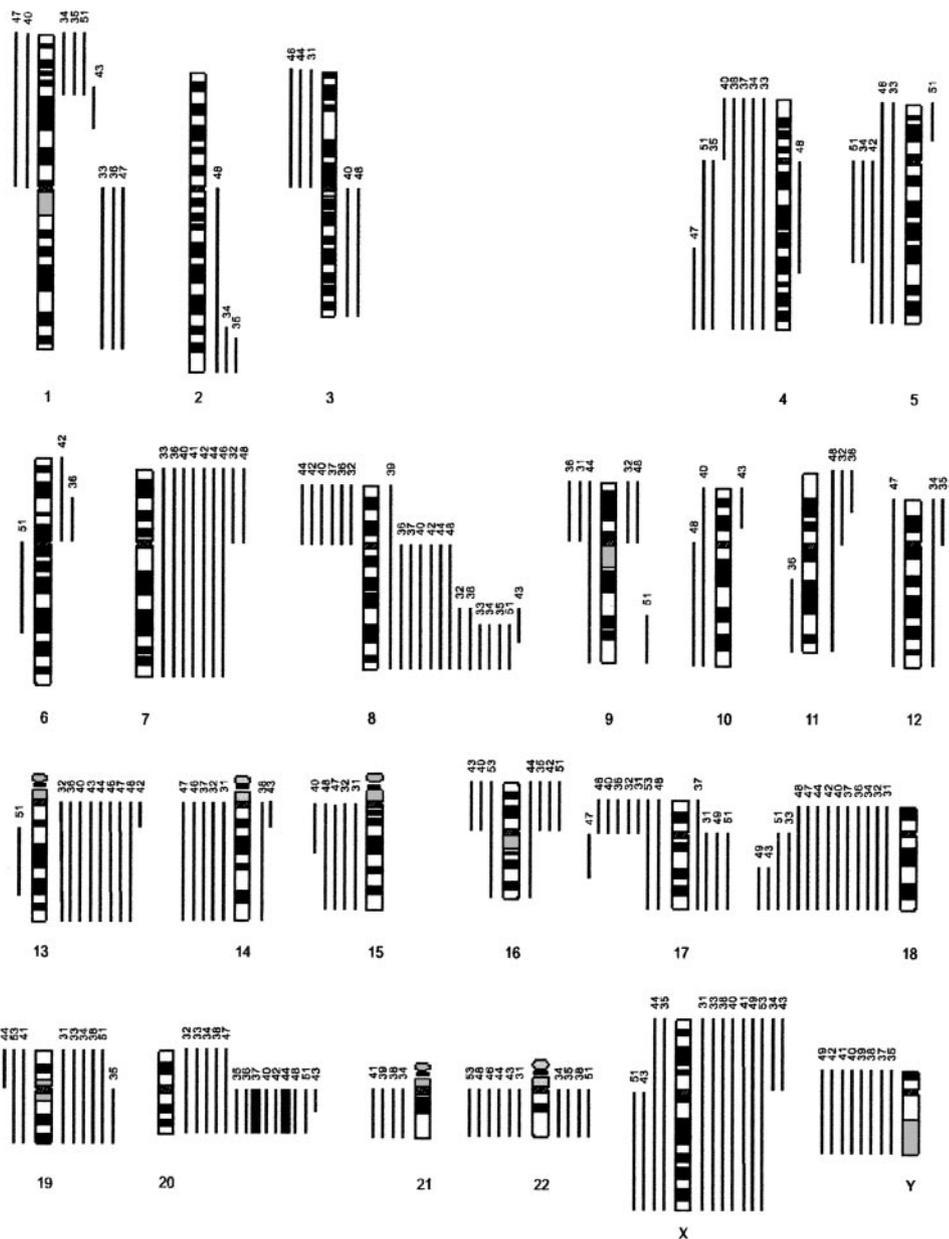


Fig. 3 Karyogram of chromosomal gains and losses in group 3 (T_{3-4} , N_{1-3}).

this type of malignancy. In this respect, the genetic analysis of primary colorectal cancers that have the capacity to form lymph node metastases *versus* cancers of the same size that do not metastasize is essential to identify the genes responsible. Furthermore, the establishment of biomarkers for molecular staging based on indicative genomic hot spots is a major goal in translational research. We therefore studied lymph node-negative *versus* lymph node-positive colorectal cancers with different degrees of infiltration into the colonic wall. Our CGH analysis revealed certain chromosomal changes that are common in colorectal cancers, such as gains of chromosome 7, 8q, 13q, 20q, and X. Frequent chromosomal losses mapped to 4, 17p, 18q, and Y. These chromosomal changes have been described previously

and are specific for colorectal cancers (3, 4, 5, 7, 8). However, in these publications, no detailed chromosomal analysis was made with respect to the nodal status.

In the present study, it is shown that lymph node-positive carcinomas reveal a very high degree of chromosomal instability compared with lymph node-negative tumors with the same depth of infiltration into the colonic wall. It was demonstrated that genomic gains and losses result in up-regulation or down-regulation of gene activity measured by expression profiling (16, 17). It can therefore be hypothesized that the metastatic phenotype requires a higher number of up-regulated oncogenes and down-regulated tumor suppressor genes. However, a study has recently been published demonstrating that chromosomal

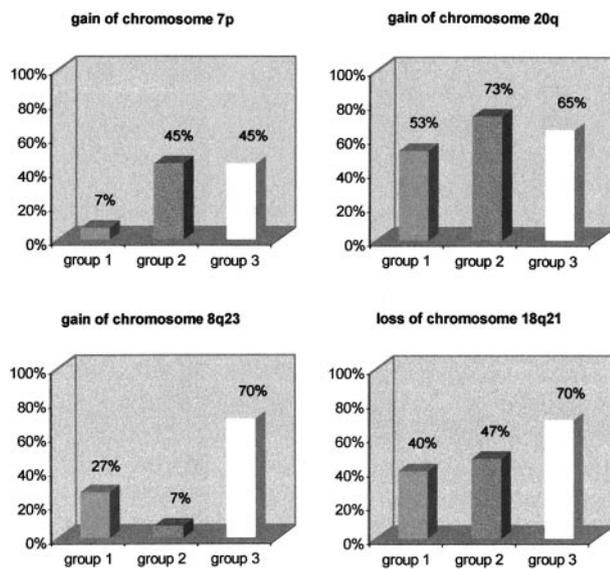


Fig. 4 Frequency of subchromosomal alterations in groups 1–3.

gains do not necessarily result in an up-regulation of genes located at the corresponding chromosomal loci in colorectal cancer (18).

The major finding of the present study is the high frequency of chromosomal gains at the 8q23–24 locus almost exclusively in lymph node-positive colorectal cancers. This chromosomal gain is rarely found in lymph node-negative carcinomas.

In addition to the data in colorectal cancers, the high frequency of gains of 8q23–24 has also been demonstrated in esophageal cancers (19). In this study, the authors conclude that gain of chromosome 8q23-ter could be used as marker to predict lymph node metastases in esophageal cancers. In our study, however, gain of 20q is also frequently found, but it does not significantly correlate with lymph node positivity. In this respect, gain of chromosome 20q seems rather to be a marker for hepatic metastases in colorectal cancers (8).

Relevant target genes mapping to the genomic region 8q23–24 are *Myc*, *EIF3S3*, *PVT 1*, *BV 1*, and the *PRL-3* gene. For instance, *EIF3S3* encodes for the p40 subunit of the eukaryotic translation initiation factor 3 (20). It has been demonstrated that amplification of *EIF3S3* is a marker of tumor progression, worse prognosis, and, in particular, lymphatic metastases in prostate cancer (21). Interphase fluorescence *in situ* hybridization analysis of colorectal cancers at different stages is therefore needed to determine the role of *EIF3S3* in lymphatic metastases. Additionally, the *PRL-3* gene has recently been demonstrated to be associated with metastasis formation in colorectal cancers and to be the possible target gene of the underlying amplification of 8q24 (22).

In summary, the study indicates that a high degree chromosomal instability correlates with colorectal cancers metastatic to the surrounding lymph nodes. In particular, gain of the chromosomal locus 8q23–24 almost exclusively occurs in lymph node-positive cancers and might pinpoint relevant target genes located in this region. Detection of gains of 8q23–24 by

using interphase DNA probes on cytological specimens or colorectal DNA chip technology in the clinical setting might predict lymph node positivity before therapy. Such molecular approaches could enhance the sensitivity and specificity of precise staging that is mandatory for individual multimodal cancer therapy.

REFERENCES

- Kapiteijn, E., Marijnen, C. A., Nagtegaal, I. D., Putter, H., Steup, W. H., Wiggers, T., Rutten, H. J., Pahlman, L., Glimelius, B., van Krieken, J. H., Leer, J. W., van de Velde, C. J., Dutch Colorectal Cancer Group. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. *N. Engl. J. Med.*, 345: 638–646, 2001.
- Ghadimi, B. M., and Schlag, P. M. Tumor metastasis. Molecular principles and therapeutic options. *Chirurg*, 69: 1315–1322, 1998.
- Ried, T., Knutzen, R., Steinbeck, R., Blegen, H., Schröck, E., Heselmeyer, K., du Manoir, S., and Auer, G. 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.
- Meijer, G. A., Hermsen, M. A., Baak, J. P., van Diest, P. J., Meuwissen, S. G., Belien, J. A., Hoovers, J. M., Joenje, H., Snijders, P. J., and Walboomers, J. M. Progression from colorectal adenoma to carcinoma is associated with non-random chromosomal gains as detected by comparative genomic hybridisation. *J. Clin. Pathol.*, 51: 901–909, 1998.
- Ghadimi, B. M., Sackett, D. L., Difilippantonio, M. J., Schröck, E., Neumann, T., Jauho, A., Auer, G., and Ried, T. Centrosome amplification and instability occurs exclusively in aneuploid, but not in diploid colorectal cancer cell lines, and correlates with numerical chromosomal aberrations. *Genes Chromosomes Cancer*, 27: 183–190, 2000.
- Knösel, T., Petersen, S., Schwabe, H., Schlüms, K., Stein, U., Schlag, P. M., Dietel, M., and Petersen I. Incidence of chromosomal imbalances in advanced colorectal carcinomas and their metastases. *Virchows Arch.*, 440: 187–194, 2002.
- Al-Mulla, F., Keith, W. N., Pickford, I. R., Going, J. J., and Birnie, G. D. Comparative genomic hybridization analysis of primary colorectal carcinomas and their synchronous metastases. *Genes Chromosomes Cancer*, 24: 306–314, 1999.
- Korn, W. M., Yasutake, T., Kuo, W. L., Warren, R. S., Collins, C., Tomita, M., Gray, J., and Waldman, F. M. Chromosome arm 20q gains and other genomic alterations in colorectal cancer metastatic to liver, as analyzed by comparative genomic hybridization and fluorescence *in situ* hybridization. *Genes Chromosomes Cancer*, 25: 82–90, 1999.
- Aragane, H., Sakakura, C., Nakanishi, M., Yasuoka, R., Fujita, Y., Taniguchi, H., Hagiwara, A., Yamaguchi, T., Abe, T., Inazawa, J., and Yamagishi, H. Chromosomal aberrations in colorectal cancers and liver metastases analyzed by comparative genomic hybridization. *Int. J. Cancer*, 94: 623–629, 2001.
- Ghadimi, B. M., Schröck, E., Walker, R. L., Wangsa, D., Jauho, A., Meltzer, P. S., and Ried, T. Specific chromosomal aberrations and amplification of the AIB1 nuclear receptor coactivator gene in pancreatic carcinomas. *Am. J. Pathol.*, 154: 525–536, 1999.
- du Manoir, S., Kallioniemi, O. P., Lichter, P., Piper, J., Benedetti, P. A., Carothers, A. D., Fantes, J. A., Garcia-Sagredo, J. M., Gerdes, T., Giollant, M., *et al.* Hardware and software requirements for quantitative analysis of comparative genomic hybridization. *Cytometry*, 19: 4–9, 1995.
- Ried, T., Heselmeyer-Haddad, K., Blegen, H., Schröck, E., and Auer, G. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. *Genes Chromosomes Cancer*, 25: 195–204, 1999.
- Frykholm, G. J., Glimelius, B., and Pahlman, L. Preoperative or postoperative irradiation in adenocarcinoma of the rectum: final treat-

- ment results of a randomized trial and an evaluation of late secondary effects. *Dis. Colon Rectum*, 36: 564–572, 1993.
14. Swedish Rectal Cancer Trial. Improved survival with preoperative radiotherapy in respectable rectal cancer. *N. Engl. J. Med.*, 336: 980–987, 1997.
15. Holen, K. D., and Saltz, L. B. New therapies, new directions: advances in the systemic treatment of metastatic colorectal cancer. *Lancet Oncol.*, 2: 290–297, 2001.
16. Bärlund, M., Monni, O., Kononen, J., Cornelison, R., Torhorst, J., Sauter, G., Kallioniemi, O. P., and Kallioniemi, A. Multiple genes at 17q23 undergo amplification and overexpression in breast cancer. *Cancer Res.*, 60: 5340–5344, 2000.
17. Monni, O., Bärlund, M., Mousses, S., Kononen, J., Sauter, G., Heiskanen, M., Paaola, P., Avela, K., Chen, Y., Bittner, M. L., and Kallioniemi, A. Comprehensive copy number and gene expression profiling of the 17q23 amplicon in human breast cancer. *Proc. Natl. Acad. Sci. USA*, 98: 5711–5716, 2001.
18. Platzer, P., Upender, M. B., Wilson, K., Willis, J., Lutterbaugh, J., Nosrati, A., Willson, J. K., Mack, D., Ried, T., and Markowitz, S. Silence of chromosomal amplifications in colon cancer. *Cancer Res.*, 62: 1134–1138, 2002.
19. Tada, K., Oka, M., Tangoku, A., Hayashi, H., Oga, A., and Sasaki, K. Gains of 8q23-qter and 20q and loss of 11q22-qter in esophageal squamous cell carcinoma associated with lymph node metastasis. *Cancer (Phila.)*, 88: 268–273, 2000.
20. Hershey, J. W., Asano, K., Naranda, T., Vornlocher, H. P., Hanachi, P., and Merrick, W. C. Conservation and diversity in the structure of translation initiation factor EIF3 from humans and yeast. *Biochimie (Paris)*, 78: 903–907, 1996.
21. Saramäki, O., Willi, N., Bratt, O., Gasser, T. C., Koivisto, P., Nupponen, N. N., Bubendorf, L., and Visakorpi, T. Amplification of *EIF3S3* gene is associated with advanced stage in prostate cancer. *Am. J. Pathol.*, 159: 2089–2094, 2001.
22. Saha, S., Bardelli, A., Buckhaults, P., Velculescu, V. E., Rago, C., St Croix, B., Romans, K. E., Choti, M. A., Lengauer, C., Kinzler, K. W., and Vogelstein, B. A. phosphatase associated with metastasis of colorectal cancer. *Science (Wash. DC)*, 294: 1343–1346, 2001.