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Chromosomal copy number changes of locally advanced rectal cancers treated with preoperative chemoradiotherapy

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Abstract

Introduction—Standard treatment of rectal cancer patients comprises preoperative chemoradiotherapy followed by radical surgery. However, clinicians are faced with the problem that response rates vary from one individual to another. Predictive biomarkers would therefore be helpful.

Materials and Methods—In order to identify genomic imbalances that might assist in stratifying tumors into responsive or non-responsive, we used metaphase comparative genomic hybridization to prospectively analyze pre-therapeutic biopsies from 42 patients with locally advanced rectal cancers. These patients were subsequently treated with 5-FU based preoperative chemoradiotherapy.

Results—Based on downsizing of the T-category, 21 rectal cancers were later classified as responsive, while 21 were non-responsive. Comparing these two groups, we could show that gains of chromosomal regions 7q32-q36 and 7q11-q31, and amplifications of 20q11-q13 were significantly associated with responsiveness to preoperative chemoradiotherapy ($P < 0.05$). However, the probability to detect these copy number changes by chance is high ($P = 0.21$).

Conclusion—Our primary results suggest that pre-therapeutic evaluation of chromosomal copy number changes may be of value for response prediction of rectal cancers to preoperative chemoradiotherapy. This will require validation in a larger cohort of patients.

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Keywords

Rectal cancer; 5-FU-based preoperative chemoradiotherapy; therapeutic response; copy number changes; response prediction

Introduction

According to the results of the CAO/ARO/AIO-94 trial of the German Rectal Cancer Study Group, preoperative 5-FU based chemoradiotherapy (CT/RT) is recommended for locally advanced rectal cancers (UICC stage II/III) in Germany, large parts of Europe and the U.S. [1]. However, clinicians face a considerable problem because the response of individual tumors to preoperative CT/RT is very heterogeneous, ranging from complete response to resistance. Consequently, phase-I/II trials have been initiated to explore whether intensifying preoperative treatment could increase the rate of complete tumor remission, which has been demonstrated to result in a pronounced survival benefit [2], and to reduce the risk of metastatic spread [3–6].

Regardless of these improvements, it obviously remains of considerable clinical interest to identify pre-therapeutic markers of response. In a previous investigation, we were able to identify a set of 54 genes that were significantly differentially expressed between responsive and non-responsive tumors [7]. Subsequently, we could show that these gene expression signatures also correlated with an increased risk of cancer recurrence [8]. Since such analyses have yet not been conducted on the DNA level, we wished to explore whether significant differences can also be detected in the tumor genomes using metaphase comparative genomic hybridization (CGH).

Materials and Methods

Selection of Patients, Study Design and Treatment

All 42 patients participated in the CAO/ARO/AIO-94 [1] or CAO/ARO/AIO-04 trial of the German Rectal Cancer Study Group, and were treated at the Department of General and Visceral Surgery, University Medicine Göttingen, Germany. Preoperative CT/RT, surgical resection and pathological workup were standardized according to the guidelines of these randomized phase-III trials.

Pre-therapeutic staging included rigid rectoscopy and endorectal ultrasound, which was performed by two experienced surgeons, colonoscopy, abdominal and pelvic computed tomography and chest X-ray. Only locally advanced adenocarcinomas (cUICC II/III) located within 12 cm from the anocutaneous verge were included. All patients subsequently received a total radiation dose of 50.4 Gy (single dose of 1.8 Gy) accompanied by a 120-hour continuous intravenous application of 5-FU (1000 mg/m²/day on days 1–5 and days 28–33). After an interval of approximately six weeks after completion of CT/RT, standardized surgery was performed including total mesorectal excision [9]. The clinical data are summarized in Table 1, and the experimental design is illustrated in Figure 1.

Ascertainment of Tumor Biopsies

From each patient, we prospectively collected pre-therapeutic biopsies from adjacent representative areas of the tumors, adhering to the guidelines set by the local ethical review board. The first one was used for histopathological confirmation of tumor diagnosis; the second one was immediately stored in RNAlater (Ambion, Austin, TX) for subsequent extraction of nucleic acids.

Classification of Response

Response was defined as downsizing of the primary tumor by comparing the pre-therapeutic T-category (determined by endorectal ultrasound) with the histopathological T-category (after surgical resection). As previously described, tumors exhibiting a T-level downsizing of at least one level were considered responsive [7,8].

Isolation of Tumor DNA and Comparative Genomic Hybridization

DNA was isolated using TRIZOL (Invitrogen, Carlsbad, CA) following standard procedures, and comparative genomic hybridization was performed as previously described [10]. The protocol can be found at <http://www.riedlab.nci.nih.gov/protocols.asp>. Briefly, 200 ng of tumor and sex-matched normal genomic DNA, nick translation labeled with biotin-16-dUTP (Roche, Mannheim, Germany) or digoxigenin-12-dUTP (Roche, Mannheim, Germany), respectively, were combined with an excess (20 µg) of the Cot-1 fraction of human DNA (Invitrogen, Carlsbad, CA) and precipitated. DNA was resuspended in a hybridization solution (50% formamide, 2xSSC, 10% dextran sulfate), denatured, pre-annealed for one hour at 37°C, and applied to pretreated and denatured slides containing normal human metaphase spreads. Hybridization was performed at 37°C in a moist chamber for 72 hours. After post-hybridization washes, tumor DNA was detected with Avidin-FITC (Vector, Burlingame, CA) and the reference DNA was detected with mouse anti-digoxigenin (Sigma, St. Louis, MO). The slides were counterstained with DAPI and embedded in an antifade solution containing para-phenylene-diamine (Sigma, St. Louis, MO).

Images were acquired for each fluorochrome using a cooled CCD camera (DFC 350 FX, Leica, Bensheim, Germany) coupled to an epifluorescence microscope (DM 6000, Leica, Bensheim, Germany) containing fluorochrome-specific filter sets. For automated karyotyping and analysis, CW-4000 imaging software (Leica, Cambridge, England) was used.

Statistical analysis: Chromosomal Imbalances and Clinical Response to Preoperative Chemoradiotherapy

To identify chromosomal loci that were differentially affected by copy number changes in responsive and non-responsive tumors, we first divided the human genome into 320 bands according to the cytogenetic regions of the International System of Cytogenetic Nomenclature [11]. The p-arms of the acrocentric chromosomes 13, 14, 15, 21 and 22, the centromeres and the entire X and Y chromosome were excluded from further analyses, leaving a final set of 260 chromosome bands. For each band we assigned a numerical value corresponding to a chromosomal loss (−1), no chromosomal change (0), chromosomal gain (+1) or amplification (+2). Clustering of those bands that exhibited exactly the same patterns of gains or losses (i.e., linkage) in the tumor samples resulted in 69 band-groups.

In order to identify chromosomal imbalances associated with response to CT/RT, we applied the Wilcoxon statistic. As a rank statistic, it arranges observations in ascending order and uses their rank instead of the actual observation value. These ranks are combined in a rank statistic, which forms the basis for further analysis of the difference between the medians of the two groups. We used a permutation method for computing the p-value for the rank statistic in lieu of the classical method, since the latter becomes problematic when there are a large number of ties between the observations. To compute the *CGH p-value* for each band-group, we repeatedly permuted the class labels (response and non-response indicators) and calculated the proportion of times the rank-statistic of the resulting dataset is more extreme than the one we obtained.

Results

We have previously reported that a set of 54 differentially expressed genes allows response prediction of rectal cancers to CT/RT with an accuracy of 83% [7], and, very recently, we were able to show that these gene expression signatures correlated with the risk to develop recurrent disease [8]. We also previously reported a linear relationship of genomic copy number with average gene expression levels [10,12,13]. We now wanted to examine whether the mechanism of transcriptional deregulation of specific genes involved in response prediction relates to genomic copy number variations as well.

Patient Characteristics

Forty-one patients were diagnosed with uT3 carcinomas, while one patient exhibited an uT4 carcinoma. Response of the 42 rectal adenocarcinomas (cUICC II, n=15 and cUICC III, n=27) to preoperative CT/RT, based on T-level downsizing, resulted in the classification of 21 prospectively collected biopsies as responders (P1–2, P4, P6–7, P24, P28, P30–31, P34, P36, P38–39, P41–43, P45–46, P48, 50–51), and the remaining 21 patients as non-responders (P10–15, P17, P20–23, P26, P29, P32–33, P35, P37, P40, P44, P47, P49) (Table 1).

Chromosomal Imbalances

In order to identify potential differences in the patterns of chromosomal gains and losses in responsive and non-responsive tumors, we analyzed all cases using CGH. The results of the individual CGH experiments are depicted in Figures 2a and 2b. Within these karyograms, lines to the left of the chromosomal ideograms indicate chromosomal losses (ratio of 0.8), lines to the right chromosomal gains (ratio of 1.2). Amplifications (ratio of > 1.5) are drawn as bold lines.

Copy number gains most frequently affected chromosome arms 7p (40%), 8q (52%), 13q (67%), 20p (38%) and 20q (67%), while frequent losses mapped to chromosome arms 8p (45%), 17p (74%) and 18q (43%). These findings are in concordance with previous reports on colorectal carcinomas [10,12–17]. For a detailed case summary, see <http://www.ncbi.nlm.nih.gov/sky/skyweb.cgi>. While only one case (P37) did not display any genomic imbalances, the remaining 41 tumors were aneuploid, with aberrations affecting between one and 18 chromosomes. Dividing the total number of chromosomal copy alterations (n=330) by the number of tumors analyzed (n=42), we obtained an average number of copy alterations (ANCA) value of 7.9 (for details see Ried et al. 1999 [18]). Amplifications were mapped to chromosome arms 8q (n=2), 13q (n=12), 20p (n=2) and 20q (n=8), as well as chromosomes 20 (n=2) and 22 (n=1). Regional amplifications were located on chromosomes 8q23-ter and 12p13.

Chromosomal Imbalances and Clinical Response to Preoperative Chemoradiotherapy

Comparing the chromosomal imbalances of 21 responders and 21 non-responders, we observed that the majority of copy number changes were present at higher frequencies in the responsive tumors (Figures 2a and 2b). To achieve a more objective measure of the genomic instability, we calculated the average number of copy alternations (ANCA). The ANCA values are 8.9 for the responders and 6.8 for the non-responders.

Using the permutation p-values for the rank-statistic, three band-groups were identified which were significantly different ($P<0.05$): 7q32-7q36 ($P=0.015$), 7q11-7q31 ($P=0.025$) and 20q11-20q13 ($P=0.04$) (Figure 3). To account for multiple testing, we calculated the probability of obtaining three band-groups with $P<0.05$ by chance. This was done by permuting the class labels (thus removing any correlation between response and gain/loss),

again calculating *CGH p-values*, and estimating the proportion of times three or more band-groups with a $P < 0.05$ were obtained. The corresponding *p-value* was determined to be $P = 0.21$. Thus we cannot reject the possibility that these three chromosomal aberrations were discovered by random chance. We were then curious to explore whether those tumors that showed a complete regression after preoperative CT/RT exhibited specific DNA aberrations. However, we could not detect significant differences between these tumors and those with a partial regression or complete remission (data not shown).

Correlation of Chromosomal Imbalances and Gene Expression Signatures

To find further evidence that these three chromosome bands differentiate responsive and non-responsive tumors, we used previously established gene expression signatures for 12 of the 42 tumors [7] to calculate a measure of the overall differential expression of the genes belonging to a particular band-group (the *gene expression p-value*). The LS statistic from the Gene-set Class Comparison tool of BRB Array-Tools was used for these analyses [19]. The LS statistic reflects the mean of the negative logarithms of the individual *p-values* for differential expression for all the genes present in the band-group [20]. A large number of genes with moderately small *p-values* for differential expression will result in a large value for this statistic, as will a small number of genes with very small *p-values*. Thus the LS statistic captures the overall correlations between the expressions of the genes present in the band-groups for response/non-response. A *p-value* for the LS statistic is derived by randomly selecting k genes from the array, and then computing the LS statistic for this group. The proportion of times an equal or higher LS statistic is obtained using this procedure is an estimate of the *p-value*, which we defined as the *gene expression p-value*.

However, insignificant *gene expression p-values* were obtained for all three band-groups, i.e. $P = 0.26$ (7q32-7q36), $P = 0.054$ (7q11-7q31) and $P = 0.77$ (20q11-20q13). Thus, the response status is not influenced by altered expression of the genes resident on those chromosomal regions that differentiate responders and non-responders (data not shown).

Discussion

Standard treatment of locally advanced rectal adenocarcinomas includes preoperative CT/RT followed by radical surgery [1]. However, the clinical responsiveness to multimodal therapy strategies ranges from complete response to resistance. A pre-therapeutic stratification of cancer patients into responders (who would benefit from standard 5-FU based CT/RT) and non-responders (who might benefit from more aggressive or alternative therapies) therefore remains of high clinical value for individualized, personalized therapy planning.

Numerous immunohistochemical studies have been conducted to predict response to preoperative CT/RT. The most frequently analyzed proteins were p53 [21–36], p27 [28,37], thymidylate synthetase [29,35,38,39], bcl-2 [23,25,26,29,30,32,34], Ki-67 [23,30,32,34,36] and PCNA [23,33,40], but the results remain contradictory.

Unfortunately, differences in the clinical evaluation of the patients in these studies make it extremely cumbersome to dissect the cause of the conflicting results. *Firstly*, different definitions of response were used, i.e., T level downsizing, reduction of the tumor diameter or tumor volume, and histomorphological regression grading. *Secondly*, tumor staging was performed with different diagnostic methods, i.e., using magnetic resonance imaging, computed tomography, endorectal ultrasound or clinical assessment. *Thirdly*, therapeutic strategies varied dramatically, i.e., some clinics used radiation alone, and some applied chemoradiotherapy with 5-FU monotherapy or 5-FU combined with oxaliplatin. Other investigators even added hyperthermia.

Previously, we investigated whether pre-therapeutic gene expression signatures exist which characterize the clinical response of rectal adenocarcinomas to preoperative CT/RT [7]. Analyzing 30 biopsies using expression microarrays, we identified a set of 54 genes that showed significantly different ($P<0.001$) expression levels between responders and non-responders. These genes have been recently shown to correlate with the development of metastatic disease in these patients [8]. In the present study, we now wished to explore whether differences between responsive and non-responsive tumors can also be observed on the DNA level. We therefore screened pre-therapeutic biopsies from 42 patients with locally advanced rectal cancers using chromosome comparative genomic hybridization (CGH). All patients participated in prospective phase-III clinical trials, and received 5-FU based preoperative CT/RT. To the best of our knowledge, this is the first study in which copy number profiles of rectal carcinomas were systematically correlated with response to preoperative treatment.

We first observed that the identified chromosomal imbalances are in concordance with previous reports on colorectal carcinomas (recently reviewed in Grade et al. 2006 [41]). Interestingly, responsive tumors revealed a higher frequency of chromosomal copy number changes, which is reflected by a higher ANCA value (8.9 compared to 6.8). When we performed a Wilcoxon rank statistic, we obtained three band-groups that were significantly differentially gained/amplified between responsive and non-responsive tumors (Figure 3): 7q32-7q36 ($P=0.015$), 7q11-7q31 ($P=0.025$) and 20q11-20q13 ($P=0.040$). However, we also calculated a p-value of $P=0.21$ for the likelihood that these aberrations were identified by chance.

To find further evidence that these three chromosomal bands represent differentiating characteristics between responsive and non-responsive tumors, we used previously obtained gene expression signatures for 12 of these 42 tumors to investigate whether these chromosomal alterations influence the clinical response by altering the expression of its resident genes. We specifically focused on these 12 tumors because corresponding gene expression profiles were available only for these patients. However, we again obtained insignificant gene expression p-values for all three band-groups, i.e., $P=0.26$ (7q32-7q36), $P=0.054$ (7q11-7q31) and $P=0.77$ (20q11-20q13), which means that responsive and non-responsive tumors show similar expression values for the genes residing on these three chromosomal regions.

In summary, we identified three chromosomal regions that exhibited different copy numbers comparing tumors that were responsive and non-responsive to preoperative chemoradiotherapy. However, there remains the possibility that these genomic copy number changes do not represent true biological differences between responsive and non-responsive tumors but artificial noise. One may speculate that the relatively small sample size precluded significant results. We believe this to be unlikely, because response prediction of rectal carcinomas to preoperative chemoradiotherapy has already been successfully performed using gene expression microarrays with smaller or similar data sets [7,42,43]. However, the data are promising enough that we plan to use high-resolution array CGH to repeat mapping of chromosomal imbalances. Integrated into a Clinical Research Unit entitled "Biological Basis of Individual Tumor Response in Patients with Rectal Cancer" (<http://www.kfo179.de>), we have therefore initiated such an analysis.

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References

1. Sauer R, Becker H, Hohenberger W, Rodel C, Wittekind C, Fietkau R, Martus P, Tschmelitsch J, Hager E, Hess CF, Karstens JH, Liersch T, Schmidberger H, Raab R. Preoperative versus postoperative chemoradiotherapy for rectal cancer. *N Engl J Med*. 2004; 351(17):1731–40. [PubMed: 15496622]
2. Rödel C, Martus P, Papadopoulos T, Füzesi L, Klimpfinger M, Fietkau R, Liersch T, Hohenberger W, Raab R, Sauer R, Wittekind C. Prognostic significance of tumor regression after preoperative chemoradiotherapy for rectal cancer. *J Clin Oncol*. 2005; 23(34):8688–96. [PubMed: 16246976]
3. Gerard JP, Chapet O, Nemoz C, Romestaing P, Mornex F, Coquard R, Barbet N, Atlan D, Adeleine P, Freyer G. Preoperative concurrent chemoradiotherapy in locally advanced rectal cancer with high-dose radiation and oxaliplatin-containing regimen: the Lyon R0-04 phase II trial. *J Clin Oncol*. 2003; 21(6):1119–24. [PubMed: 12637479]
4. Rödel C, Grabenbauer GG, Papadopoulos T, Hohenberger W, Schmoll HJ, Sauer R. Phase I/II trial of capecitabine, oxaliplatin, and radiation for rectal cancer. *J Clin Oncol*. 2003; 21(16):3098–104. [PubMed: 12915600]
5. Hofheinz RD, von Gerstenberg-Helldorf B, Wenz F, Gnad U, Kraus-Tiefenbacher U, Muldner A, Hehlmann R, Post S, Hochhaus A, Willeke F. Phase I trial of capecitabine and weekly irinotecan in combination with radiotherapy for neoadjuvant therapy of rectal cancer. *J Clin Oncol*. 2005; 23(7):1350–7. [PubMed: 15684318]
6. Rödel C, Liersch T, Hermann RM, Arnold D, Reese T, Hipp M, Furst A, Schwella N, Bieker M, Hellmich G, Ewald H, Haier J, Lordick F, Flentje M, Sulberg H, Hohenberger W, Sauer R. Multicenter phase II trial of chemoradiation with oxaliplatin for rectal cancer. *J Clin Oncol*. 2007; 25(1):110–7. [PubMed: 17194912]
7. Ghadimi BM, Grade M, Difilippantonio MJ, Varma S, Simon R, Montagna C, Füzesi L, Langer C, Becker H, Liersch T, Ried T. Effectiveness of gene expression profiling for response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy. *J Clin Oncol*. 2005; 23(9):1826–38. [PubMed: 15774776]
8. Liersch T, Grade M, Gaedcke J, Varma S, Difilippantonio MJ, Langer C, Hess CF, Becker H, Ried T, Ghadimi BM. Preoperative Chemoradiotherapy in Locally Advanced Rectal Cancer: Correlation of a Gene Expression-Based Response Signature with Recurrence. *Cancer Genet Cytogenet*. In press.
9. Heald RJ, Ryall RD. Recurrence and survival after total mesorectal excision for rectal cancer. *Lancet*. 1986; 1(8496):1479–82. [PubMed: 2425199]
10. Grade M, Hormann P, Becker S, Hummon AB, Wangsa D, Varma S, Simon R, Liersch T, Becker H, Difilippantonio MJ, Ghadimi BM, Ried T. Gene expression profiling reveals a massive, aneuploidy-dependent transcriptional deregulation and distinct differences between lymph node-negative and lymph node-positive colon carcinomas. *Cancer Res*. 2007; 67(1):41–56. [PubMed: 17210682]
11. Mitelman, F. *ISCN. An International System for Human Cytogenetic Nomenclature*. Basel: S. Karger; 1995.
12. Grade M, Ghadimi BM, Varma S, Simon R, Wangsa D, Barenboim-Stapleton L, Liersch T, Becker H, Ried T, Difilippantonio MJ. Aneuploidy-dependent massive deregulation of the cellular transcriptome and apparent divergence of the Wnt/beta-catenin signaling pathway in human rectal carcinomas. *Cancer Res*. 2006; 66(1):267–82. [PubMed: 16397240]
13. Camps J, Grade M, Nguyen QT, Hormann P, Becker S, Hummon AB, Rodriguez V, Chandrasekharappa S, Chen Y, Difilippantonio MJ, Becker H, Ghadimi BM, Ried T. Chromosomal breakpoints in primary colon cancer cluster at sites of structural variants in the genome. *Cancer Res*. 2008; 68(5):1284–95. [PubMed: 18316590]
14. Ried T, Knutzen R, Steinbeck R, Blegen H, Schrock E, Heselmeyer K, du Manoir S, Auer G. Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses

- during the genesis of colorectal tumors. *Genes Chromosomes Cancer*. 1996; 15(4):234–45. [PubMed: 8703849]
15. Meijer GA, Hermesen MA, Baak JP, van Diest PJ, Meuwissen SG, Belien JA, Hoovers JM, Joenje H, Snijders PJ, Walboomers JM. Progression from colorectal adenoma to carcinoma is associated with non-random chromosomal gains as detected by comparative genomic hybridisation. *J Clin Pathol*. 1998; 51(12):901–9. [PubMed: 10070331]
 16. Hermesen M, Postma C, Baak J, Weiss M, Rapallo A, Sciotto A, Roemen G, Arends JW, Williams R, Giaretti W, De Goeij A, Meijer G. Colorectal adenoma to carcinoma progression follows multiple pathways of chromosomal instability. *Gastroenterology*. 2002; 123(4):1109–19. [PubMed: 12360473]
 17. Ghadimi BM, Grade M, Liersch T, Langer C, Siemer A, Fuzesi L, Becker H. Gain of chromosome 8q23–24 is a predictive marker for lymph node positivity in colorectal cancer. *Clin Cancer Res*. 2003; 9(5):1808–14. [PubMed: 12738738]
 18. Ried T, Heselmeyer-Haddad K, Blegen H, Schrock E, Auer G. Genomic changes defining the genesis, progression, and malignancy potential in solid human tumors: a phenotype/genotype correlation. *Genes Chromosomes Cancer*. 1999; 25(3):195–204. [PubMed: 10379865]
 19. Simon, R.; Peng, A. BRB ArrayTools. v. 3.5.0. Bethesda: National Cancer Institute; 2007.
 20. Pavlidis P, Qin J, Arango V, Mann JJ, Sibille E. Using the gene ontology for microarray data mining: a comparison of methods and application to age effects in human prefrontal cortex. *Neurochem Res*. 2004; 29(6):1213–22. [PubMed: 15176478]
 21. Spitz FR, Giacco GG, Hess K, Larry L, Rich TA, Janjan N, Cleary KR, Skibber JM. p53 immunohistochemical staining predicts residual disease after chemoradiation in patients with high-risk rectal cancer. *Clin Cancer Res*. 1997; 3(10):1685–90. [PubMed: 9815551]
 22. Luna-Perez P, Arriola EL, Cuadra Y, Alvarado I, Quintero A. p53 protein overexpression and response to induction chemoradiation therapy in patients with locally advanced rectal adenocarcinoma. *Ann Surg Oncol*. 1998; 5(3):203–8. [PubMed: 9607618]
 23. Tannapfel A, Nusslein S, Fietkau R, Katalinic A, Kockerling F, Wittekind C. Apoptosis, proliferation, bax, bcl-2 and p53 status prior to and after preoperative radiochemotherapy for locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys*. 1998; 41(3):585–91. [PubMed: 9635706]
 24. Sakakura C, Koide K, Ichikawa D, Wakasa T, Shirasu M, Kimura A, Taniguchi H, Hagiwara A, Yamaguchi T, Inazawa J, Abe T, Takahashi T, Otsuji E. Analysis of histological therapeutic effect, apoptosis rate and p53 status after combined treatment with radiation, hyperthermia and 5-fluorouracil suppositories for advanced rectal cancers. *Br J Cancer*. 1998; 77(1):159–66. [PubMed: 9459162]
 25. Scott N, Hale A, Deakin M, Hand P, Adab FA, Hall C, Williams GT, Elder JB. A histopathological assessment of the response of rectal adenocarcinoma to combination chemo-radiotherapy: relationship to apoptotic activity, p53 and bcl-2 expression. *Eur J Surg Oncol*. 1998; 24(3):169–73. [PubMed: 9630854]
 26. Qiu H, Sirivongs P, Rothenberger M, Rothenberger DA, Garcia-Aguilar J. Molecular prognostic factors in rectal cancer treated by radiation and surgery. *Dis Colon Rectum*. 2000; 43(4):451–9. [PubMed: 10789738]
 27. Elsalem H, Robbins P, Joseph D, Powell B, Grieco F, Menso L, Iacopetta B. Can p53 alterations be used to predict tumour response to pre-operative chemo-radiotherapy in locally advanced rectal cancer? *Radiother Oncol*. 2000; 56(2):239–44. [PubMed: 10927144]
 28. Esposito G, Pucciarelli S, Alaggio R, Giacomelli L, Marchiori E, Iaderosa GA, Friso ML, Toppan P, Chieco-Bianchi L, Lise M. P27kip1 expression is associated with tumor response to preoperative chemoradiotherapy in rectal cancer. *Ann Surg Oncol*. 2001; 8(4):311–8. [PubMed: 11352304]
 29. Okonkwo A, Musunuri S, Talamonti M, Benson A 3rd, Small W Jr, Stryker SJ, Rao MS. Molecular markers and prediction of response to chemoradiation in rectal cancer. *Oncol Rep*. 2001; 8(3):497–500. [PubMed: 11295069]

30. Kim NK, Park JK, Lee KY, Yang WI, Yun SH, Sung J, Min JS. p53, BCL-2, and Ki-67 expression according to tumor response after concurrent chemoradiotherapy for advanced rectal cancer. *Ann Surg Oncol*. 2001; 8(5):418–24. [PubMed: 11407516]
31. Abe T, Sakaguchi Y, Ohno S, Ikeda Y, Kitamura K, Maehara Y, Sugimachi K. Apoptosis and p53 overexpression in human rectal cancer; relationship with response to hyperthermo-chemo-radiotherapy. *Anticancer Res*. 2001; 21(3C):2115–20. [PubMed: 11501834]
32. Roedel C, Grabenbauer GG, Hohenberger W, Papadopoulos T, Sauer R. Phase I/II trial of preoperative chemoradiotherapy with capecitabine and oxaliplatin in locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys*. 2003; 57(2 Suppl):S178.
33. Diez M, Ramos P, Medrano MJ, Muguerza JM, Villeta R, Lozano O, Escribano J, Nogueras F, Ruiz A, Granell J. Preoperatively irradiated rectal carcinoma: analysis of the histopathologic response and predictive value of proliferating cell nuclear antigen immunostaining. *Oncology*. 2003; 64(3):213–9. [PubMed: 12697960]
34. Rau B, Sturm I, Lage H, Berger S, Schneider U, Hauptmann S, Wust P, Riess H, Schlag PM, Dorken B, Daniel PT. Dynamic expression profile of p21WAF1/CIP1 and Ki-67 predicts survival in rectal carcinoma treated with preoperative radiochemotherapy. *J Clin Oncol*. 2003; 21(18):3391–401. [PubMed: 12885834]
35. Saw RP, Morgan M, Koorey D, Painter D, Findlay M, Stevens G, Clarke S, Chapuis P, Solomon MJ. p53, deleted in colorectal cancer gene, and thymidylate synthase as predictors of histopathologic response and survival in low, locally advanced rectal cancer treated with preoperative adjuvant therapy. *Dis Colon Rectum*. 2003; 46(2):192–202. [PubMed: 12576893]
36. Jakob C, Liersch T, Meyer W, Becker H, Baretton GB, Aust DE. Predictive value of Ki67 and p53 in locally advanced rectal cancer: correlation with thymidylate synthase and histopathological tumor regression after neoadjuvant 5-FU-based chemoradiotherapy. *World J Gastroenterol*. 2008; 14(7):1060–6. [PubMed: 18286688]
37. Gunther K, Dimmler A, Rodel F, Reulbach U, Merkel S, Bittorf BR, Matzel KE, Papadopoulos T, Hohenberger W, Sauer R, Rodel C. P27 does not predict histopathological response to radiochemotherapy in rectal cancer. *J Surg Res*. 2003; 113(2):179–88. [PubMed: 12957127]
38. Jakob C, Aust DE, Meyer W, Baretton GB, Schwabe W, Hausler P, Becker H, Liersch T. Thymidylate synthase, thymidine phosphorylase, dihydropyrimidine dehydrogenase expression, and histological tumour regression after 5-FU-based neo-adjuvant chemoradiotherapy in rectal cancer. *J Pathol*. 2004; 204(5):562–8. [PubMed: 15538739]
39. Jakob C, Liersch T, Meyer W, Baretton GB, Hausler P, Schwabe W, Becker H, Aust DE. Immunohistochemical analysis of thymidylate synthase, thymidine phosphorylase, and dihydropyrimidine dehydrogenase in rectal cancer (cUICC II/III): correlation with histopathologic tumor regression after 5-fluorouracil-based long-term neoadjuvant chemoradiotherapy. *Am J Surg Pathol*. 2005; 29(10):1304–9. [PubMed: 16160472]
40. Willett CG, Warland G, Cheek R, Coen J, Efird J, Shellito PC, Compton CC. Proliferating cell nuclear antigen and mitotic activity in rectal cancer: predictor of response to preoperative irradiation. *J Clin Oncol*. 1994; 12(4):679–82. [PubMed: 7908689]
41. Grade M, Becker H, Liersch T, Ried T, Ghadimi BM. Molecular cytogenetics: Genomic imbalances in colorectal cancer and their clinical impact. *Cell Oncol*. 2006; 28(3):71–84. [PubMed: 16823176]
42. Kim IJ, Lim SB, Kang HC, Chang HJ, Ahn SA, Park HW, Jang SG, Park JH, Kim DY, Jung KH, Choi HS, Jeong SY, Sohn DK, Kim DW, Park JG. Microarray gene expression profiling for predicting complete response to preoperative chemoradiotherapy in patients with advanced rectal cancer. *Dis Colon Rectum*. 2007; 50(9):1342–53. [PubMed: 17665260]
43. Rimkus C, Friederichs J, Boulesteix AL, Theisen J, Mages J, Becker K, Nekarda H, Rosenberg R, Janssen KP, Siewert JR. Microarray-based prediction of tumor response to neoadjuvant radiochemotherapy of patients with locally advanced rectal cancer. *Clin Gastroenterol Hepatol*. 2008; 6(1):53–61. [PubMed: 18166477]

Figure 1. Pictorial presentation of study design. CT/RT, chemoradiotherapy; TME, total mesorectal excision.

Figure 2.

CGH profiles of 21 responsive (A) and 21 non-responsive rectal cancers (B). Genomic copy number losses are indicated as bars to the left of the chromosome, while bars to the right of the chromosome represent copy number gains. Thick bars indicate chromosomal amplifications.

Figure 3.
Frequency of distinct sub-chromosomal alterations comparing responsive and non-responsive tumors.

Table 1

Clinical data of 42 patients. uT, pre-therapeutic T-category determined by endorectal ultrasound; ypT, T-category determined by histopathological assessment after preoperative chemoradiotherapy; uN, lymph node status by endorectal ultrasound; ypN, lymph node status by histopathological assessment; ypN total, total number of analyzed lymph nodes; ypN infiltrated, number of infiltrated lymph nodes; ypGrading, tumor grading by histopathological assessment; R, resectability (surgical resection margins); cUICC, clinical UICC stage; ypUICC, post-treatment UICC stage; UICC, International Union Against Cancer; Tis, tumor in situ.

Tumor Samples	Sex	Age	uT	ypT	Response	uN	ypN	ypN total	ypN infiltrated	ypGrading	R	cUICC	ypUICC
P1	M	61	3	0	+	0	0	18	0	x	0	II	0
P2	M	61	3	0	+	1	0	27	0	x	0	III	0
P4	M	68	3	2	+	1	0	22	0	2	0	III	I
P6	M	65	3	2	+	1	0	24	0	2	0	III	I
P7	M	49	3	1	+	0	0	18	0	2	0	II	II
P10	M	53	3	3b	-	1	1	30	1	2	0	III	III
P11	M	64	3	3b	-	1	0	15	0	2	0	III	II
P12	M	55	3	3b	-	0	0	8	1	3	0	II	III
P13	F	70	3	3b	-	1	1	27	1	2	0	III	III
P14	M	58	3	4a	-	1	1	19	1	2	0	III	III
P15	M	53	3	3b	-	1	0	28	0	2	0	III	II
P17	M	78	3	3b	-	1	1	19	2	3	0	III	III
P20	M	58	3	3c	-	0	0	16	0	2	0	II	II
P21	F	59	3	3c	-	1	0	17	0	2	0	III	II
P22	M	62	3	3a	-	1	0	14	0	2	0	III	II
P23	F	40	3	4a	-	1	1	22	1	2	0	III	III
P24	M	68	3	2	+	0	0	16	0	2	0	II	I
P26	M	62	3	3	-	1	0	20	0	2	0	III	II
P28	M	59	3	Tis	+	1	1	17	1	3	0	III	III
P29	F	68	3	3	-	1	0	15	0	2	0	III	II
P30	M	71	3	2	+	0	0	12	0	2	0	II	I
P31	M	63	3	1	+	1	0	24	0	2	0	III	I
P32	M	50	3	3d	-	0	0	34	0	2	0	II	II
P33	F	58	3	3a	-	0	0	12	0	2	0	II	II
P34	M	68	3	2	+	1	0	12	0	2	0	III	I

Tumor Samples	Sex	Age	uT	ypT	Response	uN	ypN	ypN total	ypN infiltrated	ypGrading	R	cIUCC	ypUICC
P35	M	62	3	3	—	0	0	26	0	2/3	0	II	II
P36	M	66	3	0	+	1	0	19	0	X	0	III	0
P37	M	61	3	4	—	1	2	22	6	2	0	III	III
P38	M	57	3	2	+	0	0	24	0	2	0	II	I
P39	M	70	4	3c	+	1	2	25	5	2	0	III	III
P40	M	73	3	3a	—	1	1	31	1	2	0	III	III
P41	M	59	3	2	+	1	0	5	0	2	0	III	I
P42	M	64	3	1	+	1	0	37	0	1/2	0	III	I
P43	F	48	3	1	+	1	0	47	0	1/2	0	III	I
P44	M	50	3	3b	—	0	0	26	0	2/3	0	II	II
P45	M	70	3	2	+	0	0	22	0	2/3	0	II	I
P46	F	71	3	2	+	0	0	30	0	2	0	II	I
P47	F	67	3	3b	—	0	1	15	2	2	0	II	III
P48	M	52	3	2	+	1	0	18	0	2	1	III	II
P49	M	70	3	3c	—	1	1	37	1	3	0	III	III
P50	F	53	3	1	+	1	0	20	0	2	0	III	I
P51	M	65	3	0	+	0	0	30	0	X	0	II	0

Table 2

Chromosomal gains and losses of 42 locally advanced rectal cancers. The suffix ++ indicates chromosomal amplification.

Tumor Samples	Chromosomal gains	Chromosomal losses
P1	5p, 7p, 8q, 13q21-ter, 20p, Xq	6p22-ter, 10p, 15, 17, 18p
P2	13, 20q	14, 17p, 18q
P4	20q	-
P6	7, 13, 20q++	4, 8p, 18q
P7	1q, 3q, 7p, 7q11-31, 13++, 20p, 20q++	8p, 11q, 14, 15, 18
P10	2q32-ter, 8q23-ter++, 9, 20p, 20q++	4, 5, 6q, 8p, 15, 17, 18q
P11	8q, 13	6p, 8p, 9q22-ter, 10p, 15, 17, 20, 21
P12	-	17
P13	4	17, 20q
P14	8q, 13	10p, 12p, 12q23-ter, 17
P15	1q, 7p, 9q, 13, 16, 19, 20	4, 18q
P17	9q, 20q	9p
P20	5p, 8q, 13, 20	8p, 9p, 10p, 18q22-ter, 19p
P21	7, 8q, 13, 20q	8p, 12q24.3-ter, 14, 15, 17p, 18p, 21q22
P22	1q, 2, 3, 5, 6p, 8, 12, 13++, 14, 20p++, 20q, 21, Xp	17, 18p
P23	1q, 4q, 9, 12p13++, 13++, 20, Xq	1p, 6q11-16, 11, 14, 17, 18
P24	6p11-21.3, 8q, 13, 17q, 20q++	17p
P26	7p, 13, Xp, Xq11-21	17p, 18q
P28	7, 13++, 20q++	4q, 9, 10p, 14, 15, 18, 20p
P29	-	17
P30	1q, 2, 3q, 6, 7, 8q, 9, 11, 12p12-12q14, 13++, 14, 20, X	8p, 12q23-ter, 15, 17, 18
P31	2, 5p, 6q, 7, 8q, 11, 13++, 20p, 20q++, X	5q, 8p, 17p, 18
P32	2q22-34, 8q, 11, 13++, 22++, X	8p, 17, 18q21-ter
P33	3, 5p, 6, 7p, 8q, 9, 11, 12q14-22, 13++, 20p, 20q++, Xq	8p, 17p, 18, 19, 22
P34	6q11-22, 8q, 13, X	17, 18p, 22
P35	8q, 20	8p, 15, 18q
P36	1q, 2p, 4q32-ter, 7, 12p, 13	1p, 2q, 4p, 4q11-31.3, 17p, 18, 22
P37	-	-
P38	2q23-33, 6p12-q23, 8q, 10q21-22, 11, 13	17
P39	2, 8q, 11, 13++, 20++, X	8p, 17, 18q
P40	5q14-23, 6q11-22, 7p, 8q++, 9p, 11, 12p, 13++, 20, X	8p, 15, 17p, 18q, 22
P41	4, 5, 8q, 13q21-ter, 20p++, 20q, X	6p, 14, 17, 18q, 22
P42	2, 8q, 13q21-ter, 20p, Xq	8p, 17p, 21
P43	7, 8q, 13, 20q	8p, 17p, 18, 21q22
P44	6q11-22, 7, 8q, 13q21-ter, 20q, X	8p, 17, 18q12-ter, 21q22, 22q12-ter
P45	5p, 7, 8q, 13++, 19p, 20++, X	5q, 15, 17, 18q
P46	2, 7, 8q++, 13, 20q++	8p, 17p, 18, 22
P47	1q, 8q, 12p	8p, 10q22-ter, 17

Tumor Samples	Chromosomal gains	Chromosomal losses
P48	13, 20q, X	17p, 18q22-ter
P49	1q, 5q, 7q11-31, 12q, 13q++, 20q, X	8p21-ter, 18q21-ter
P50	1q, 7, 8q, 13q++, 19, 20q	4, 8p, 15, 17p, 18
P51	1q22-ter, 3q, 7, 8q, 9p, 13, 20, X	8p, 14, 17p, 18q