Alteration of DNA Ploidy Status and Cell Proliferation Induced by Preoperative Radiotherapy Is a Prognostic Factor in Rectal Cancer

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ABSTRACT

To identify predictors of prognosis after preoperative radiotherapy, DNA ploidy and cell proliferation were investigated in 116 patients with rectal cancer. For flow cytometry, a nuclear suspension was prepared by peptic digestion of paraffin samples of biopsies taken before preoperative radiotherapy (15 × 2 Gy) and also of the resected rectal tumors after radiotherapy. The median follow-up period was 6 years. The proportion of tumor necrosis was evaluated in histological sections before and after irradiation. There was a significant decrease (74 to 48%) in aneuploid tumors after radiation. Of 86 patients with aneuploidy biopsies, 28 revealed no reduction in the proportion of aneuploid tumor cells [group AN(=/↑)], and 58 showed a reduction (mean 48.9%) or complete elimination of aneuploid tumor cells [group AN(↓/2)]). The incidence of local or distal failure was significantly reduced in the group AN(↓/2) (7.8%/20%), compared with the group AN (=/↑) (27%/54%) and the group of constant diploid tumors (n = 22; 13.6%/31.8%; P = 0.034). There was a trend of decreased recurrence rate in diploid tumors with a reduced fraction of cells in S-phase after radiotherapy. Survival was significantly increased in group AN(↓/2) (P < 0.00001). In a multivariate regression analysis, variables of independent prognostic significance were increased proportion of necrosis after irradiation and DNA ploidy group and the postoperative tumor stage. These results suggest that alterations in tumor DNA ploidy and cell proliferation induced by preoperative radiotherapy might help to identify patients likely to benefit from preoperative radiation in rectal cancer.

INTRODUCTION

Cancer of the rectum is a common visceral tumor that for decades has been managed primarily by surgery alone. Survival and local recurrence rates in rectal carcinoma after curative surgical resection have remained static for many years. Recent studies, evaluating pre- and postoperative radiotherapy with cumulative doses between 25 and 50 Gy, could demonstrate a significant reduction in local recurrence and (1, 2), in combining postoperative radiotherapy with chemotherapy, an increased survival (3). When comparing pre- and postoperative radiotherapy modalities at similar doses, preoperative radiotherapy appears to be more efficient in reducing local failure rate (4), and recently, one study has shown improved survival over surgery alone (5).

Several studies have been reported on flow cytometric DNA measurement in human tumor biopsies to obtain prognostic data from tumor ploidy and cell proliferation. In head and neck (6), cervix (7), and bladder (8) cancers, it has been suggested that tumors with an aneuploid DNA content might dispose of higher radiosensitivity than diploid tumors. As an impact of preoperative radiotherapy, the prevalence of DNA aneuploidy was significantly lower in irradiated esophagus carcinomas compared with nonirradiated cases (9). Furthermore, radiobiological investigations have shown that cell proliferation influences the radiosensitivity of tumor cells considerably (10). Tumors with a higher proliferative activity show a higher response to radiotherapeutic treatment. Thus, the aim of the current study was to determine whether flow cytometric analysis of DNA ploidy and cell proliferation and their changes induced by radiotherapy are of prognostic value in preoperatively irradiated rectal cancer.

MATERIALS AND METHODS

Patients and Tissue Samples. One hundred and sixteen patients (51 men and 65 women) with a histologically proven localized adenocarcinoma of the rectum, treated by curative resection after preoperative irradiation at the Department of Surgery, Evangelic Hospital of Düsseldorf in Germany between 1980 and 1988 were included in this study. Mean age of the patients was 64.5 years (range, 38–87 years). The preoperative radiation therapy consisted of a total dose of 30 Gy delivered to the midplane of the pelvis through opposing anterior-posterior fields given in a daily fraction of 2 Gy, according to the regimen of a randomized trial conducted by the European Organization for Research and Treatment of Cancer (1), for tumors within 10 cm of the anal verge, and additional perineal field was used for irradiation. After an average time of 14.2 ± 9.8 days, 76 and 24% of the patients underwent anterior and abdomino-perineal resection, respectively. Table 1 shows the postoperative tumor characteristics. All pathological diagnosis and classification of
Prognostic Value of Ploidy Alterations after Radiation

The number of paraffin blocks was 1.2 blocks to decrease nuclear debris (14). The average proportion of tumor sections were cutoff from the blocks. Afterward, 150–200...

For these investigations, the whole tumor tissue was available in formalin-fixed, paraffin-embedded blocks. The average number of biopsies before irradiation taken from different tumor regions during endoscopy was 4.7 ± 1.0, whereas the average number of paraffin blocks was 1.2 ± 0.6. The mean number of blocks embedding the resected tumor was 2.5 ± 0.9. Follow-up data were obtained from the charts of the patients, and the patients’ data were stored in a follow-up computer program.

Flow Cytometry. Formalin-fixed, paraffin-embedded tumor tissue was prepared and stained for flow cytometric analysis according to the modified procedure described by Hedley et al. (13). To enrich the proportion of tumor cells, the localized tumor region was microdissected from stroma tissue in paraffin blocks. Afterward, 150–200-μm sections were cutoff from the blocks to decrease nuclear debris (14). The average proportion of the tissue sample occupied by the resected tumor was 20% (range, 7–80%), whereas the analyzed proportion of the tumor biopsies before radiation was approximately 30–40%. Sections were treated with xylene over a period of 24 h to remove the paraffin and then rehydrated in ethanol and washed with distilled water. To prepare a nuclear suspension, sections were incubated in a 0.5% pepsin solution (pH 1.5, 37°C) for 90 min. After filtration through a 50-μm nylon mesh, nuclei were washed twice in PBS and then centrifuged. The nuclear pellet was resuspended in a 0.1% NP40-trisodium citrate solution (15) and treated with RNase solution (40 μg/ml). Finally the nuclear DNA was stained with propidium iodide (50 μg/ml) to analyze on a flow cytometer (Becton Dickinson, San Jose, CA), equipped with an argon laser using excitation at 488 nm. A total of 10,000 cells were used for cell cycle analysis using the Cellfit Software (Becton Dickinson, San Jose, CA).

The flow cytometric parameters evaluated included DNA ploidy, the fractions of the cell cycle compartments, and the DI (where DI represents the ratio of the aneuploid G1-G0-DNA peak channel to the diploid G1-G0-DNA peak channel). Samples were defined as aneuploid only if there was more than one G0-G1 peak in the DNA histogram. In these tumors, the first peak on the left of the histogram was considered to represent diploid G0-G1 cells. For multiple paraffin blocks, the mean values of the S-phase fraction, the DI and the aneuploid G1-G0 peak were used. Evaluation of S-phase fraction was not possible in 20 aneuploid tumors because of overlapping of the diploid G0-M cells in the channel area of the aneuploid S-phase cells in the histogram. The mean coefficient of variation of the G1 peak in this study was 6.5 ± 1.8%.

Quantification of Necrosis. The extent of necrosis in tumors was evaluated semiquantitatively by histopathological examination of all H&E-stained sections of every paraffin block of the biopsies and the resected tumors by one observer without knowledge of the patients’ outcome. The proportion of necrosis was classified in 5% gradation (ex: 0, 5, 10, and 15–100%). For the biopsy necrosis gradation, it was necessary to start with 2%, indicating a minimal proportion of necrosis. The mean number of biopsies on one histological section was 3.9 ± 1.6 with an average of 1.2 ± 0.6 sections. Because the resected tumors were fixed in a mean number of 2.5 ± 0.9 paraffin-embedded blocks, 2.5 ± 0.9 sections of each tumor were available. The mean values of the proportion of necrosis in all histological sections before and after irradiation were used for each tumor.

Statistical Analysis. Possible associations between flow cytometric results and clinicopathological characteristics were determined using the χ2 test. Yates correction was applied for a number of cases between 40 and 60 in a four-field table. Differences between mean values were analyzed using the Student’s t test. Data analysis was performed with the SPSS statistical software package (SPSS Inc., Chicago, IL). The Kaplan-Meier product-limit method was used to estimate survival or disease-free probabilities, with statistical interference’s on actuarial curves made using the Breslow and the log-rank test.

Patients dying as a result of postoperative complications (within 30 days) were excluded from the survival analysis. All patients with incomplete or missing follow-up had to be excluded for the calculations of local or distant recurrences. To determine independent prognostic factors, multivariate analysis was calculated using the Cox proportional hazards regression model.

### Table 1 Tumor characteristics of irradiated adenocarcinomas of the rectum (n = 116)

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor location from anal verge</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 to &lt;7 cm</td>
<td>49</td>
<td>42.2</td>
</tr>
<tr>
<td>7 to &lt;12 cm</td>
<td>53</td>
<td>45.7</td>
</tr>
<tr>
<td>≥12 cm</td>
<td>14</td>
<td>12.1</td>
</tr>
<tr>
<td>Grading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G1/2</td>
<td>95</td>
<td>81.9</td>
</tr>
<tr>
<td>G3</td>
<td>21</td>
<td>18.1</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3 cm</td>
<td>69</td>
<td>59.5</td>
</tr>
<tr>
<td>3–6 cm</td>
<td>41</td>
<td>35.3</td>
</tr>
<tr>
<td>&gt;6 cm</td>
<td>5</td>
<td>4.3</td>
</tr>
<tr>
<td>Tumor invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT1/2</td>
<td>98</td>
<td>84.4</td>
</tr>
<tr>
<td>pT3/4</td>
<td>18</td>
<td>15.5</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pN0</td>
<td>77</td>
<td>66.4</td>
</tr>
<tr>
<td>pN1/2</td>
<td>39</td>
<td>33.6</td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>69</td>
<td>59.5</td>
</tr>
<tr>
<td>II/III</td>
<td>47</td>
<td>40.5</td>
</tr>
</tbody>
</table>

Notes: The abbreviations used are: TNM, Tumor-Node-Metastasis; AN( / ), group of tumors with a reduced or eliminated proportion of aneuploid cells after radiotherapy; AN( =/ ), group of tumors with a constant or increased proportion of aneuploid cells after radiotherapy; DIDI, group of tumors with diploidy before and after radiotherapy; DI, DNA index; PCNA, proliferative cell nuclear antigen; SPF, S-phase fraction.

2 The abbreviations used are: TNM, Tumor-Node-Metastasis; AN( / ), group of tumors with a reduced or eliminated proportion of aneuploid cells after radiotherapy; AN( =/ ), group of tumors with a constant or increased proportion of aneuploid cells after radiotherapy; DIDI, group of tumors with diploidy before and after radiotherapy; DI, DNA index; PCNA, proliferative cell nuclear antigen; SPF, S-phase fraction.
are presented as mean ± SD. Only \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**Ploidy and S-Phase Analysis.** There are no reports available associating the DNA ploidy and preoperative radiotherapy as a prognostic tool. Speculations have been made that changes in ploidy status induced by radiation may be a useful predictor for clinical outcome in various cancers. In this study, we analyzed DNA ploidy and their alterations before and after irradiation of each tumor and compared those findings with the clinical follow-up. In the preirradiated biopsies, DNA aneuploidy was detected in 86 of 116 (74%) tumors. After radiotherapy, however, the number of aneuploid tumors decreased to 56 (48%; \( \chi^2 = 16.33; P < 0.0001 \)). The distribution of the DI was similar before and after radiation. Compared with a mean DI of 1.67 ± 0.28 in the preirradiated hyperdiploid biopsies, the mean DI in the irradiated resected aneuploid tumors decreased to 1.55 ± 0.26, being clustered around triploid levels. After radiotherapy, 24 tumors (28%) showed a decrease in the proportion of aneuploid cells compared with the pretreatment values (mean reduction, 49.8%; range, 16.7–92.2%). A representative analysis of the data is given in Fig. 1. In contrast, 28 (33%) preirradiated aneuploid tumors revealed a constant or increased proportion of aneuploid cells after radiotherapy [mean increase, 64%; range, 0–262%; group AN(↑)]. Of these 52 tumors with a decrease or increase in the amount of aneuploid tumor cells, 35 tumors (67%) consistently showed this alteration among all analyzed samples in the posttreatment assessment. However, for the rest (33%), the decrease or increase was consistent in two of three samples. Thirty-four tumors (39%) even demonstrated a complete elimination of aneuploid cells [group AN(↓)]. The percentage of tetraploid pretreatment tumors (DI, 1.8–2.2) was significantly higher in the group of tumors with an eliminated aneuploidy (41.2%) after radiotherapy than in the remaining aneuploid tumors (17.3%; \( P = 0.042 \)). From 30 preirradiated diploid tumors, nearly all (26 of 30; 87%) remained diploid (group DIDI).

Tumor cell proliferation was determined by measuring the SPF. All 26 constant diploid cases and 32 of 52 pre- and posttreatment aneuploid cases (61%) provided analyzable S-phase data. Of the 26 constant diploid tumors, 17 (65%) revealed a constant or increased SPF [S-phase (↑)] with a mean increase of 73% (range, 12–430%). Only 9 tumors (35%) showed a decreased SPF [S-phase (↓)] in the posttreatment samples [mean decrease, 44% (range, 5 to 71%)]. The mean pretreatment SPF in group S-phase (↓) (10.4 ± 2.4%) was significantly higher than in the group S-phase (↑) (5.9 ± 2.3%; \( P < 0.0001 \)).

Compared with a mean S-phase fraction of 7.4% (median, 6.8 ± 3.1%) for all diploid cases before treatment, the 32 aneuploid cases showed a significantly higher mean value of 19.5% (median, 20.3 ± 6.1%; \( P < 0.0001 \)). Fourteen aneuploid tumors (44%) revealed a constant or increased SPF (mean

![Fig. 1 DNA flow cytometric histograms of the biopsy before and the corresponding resected specimen after preoperative radiotherapy in rectal cancer. Both samples are classified as aneuploid because of a separate peak, representing the aneuploid G1 population with a DI of 1.66. The proportion of the aneuploid G1 population is reduced from 46% in the pretreatment biopsy to 11.4% in the resected tumor after irradiation. A, biopsy; B, resected tumor.)
increase, 43.3%; range, 0–99%). In 18 aneuploid tumors (56%), a mean decrease of 31.3% (range, 6.6–62%) was observed. The pretreatment fraction of the S-phase was significantly higher in the group of tumors with a reduced SPF after irradiation (23 ± 3.4% versus 15 ± 5.5%; P < 0.0001).

Characterization of Necrosis in Tumors. In biopsies, the overall percentage of necrosis was 1.6%. In the resected postirradiated tumors, the mean proportion of necrosis increased to 9.3%. The ploidy groups showed a visible difference in the increase of necrosis after radiotherapy. The group of tumors with a reduced or eliminated aneuploidy after irradiation [group AN (1/2)] showed the highest increase of necrosis with a mean of 10.9%, followed by the group AN (=1/1) with an increase of 6.2%. The lowest increase was observed in the group of constant diploid tumors (group DIDI; mean, 3.4%).

There was no significant relationship between the two ploidy groups [AN (1/2); AN (=1/1)] and clinicopathological characteristics, such as sex, age, localization, tumor differentiation, or lymph node metastasis. The association with the invasion of the primary tumor was of borderline statistical significance (P = 0.07). A decrease or elimination in the proportion of aneuploid cells is related to a significant increase of tumor necrosis (P = 0.0001).

Correlation with Clinical Follow-Up. During follow-up, 28 patients (27.2%) developed distant metastases, 10 relapsed locally (9.7%), and 4 relapsed locally and distally (3.9%). In total, the incidence of local recurrence was 13.5% (n = 14). Table 2 shows the recurrence rates in relation to different ploidy groups, pretreatment ploidy, pretreatment hyperdiploid DIs, pretreatment SPF, and changes in the SPF after irradiation. Although the pretreatment ploidy status was of no influence on recurrence, however, the changes in the proportion of aneuploidy induced by radiotherapy showed a significant impact on the number of local as well as distant recurrences. The preirradiated near-diploid tumors (DI < 1.3) relapsed distally in >50%, compared with 37.2% in the group of peri-/triploid tumors (DI, 1.3 to <1.8) and only 5% in the group of peri-/tetraploid tumors (DI, >1.8–2.2; P < 0.004). There was no significant influence on local or distant outcome between the different percentage of S-phase in the pretreatment diploid tumors, but for the alterations in the S-phase fraction after radiotherapy, a significant difference in the number of distant metastases could be observed (Table 2). In contrast, a pretreatment SPF of >20.3% in the aneuploid tumors showed a visible significant decreased overall recurrence rate.

Correlation with Patients’ Survival. Forty-four patients (40.4%) died because of cancer-related causes, and 13 (11.9%) died because of intercurrent diseases. After exclusion of postoperative deaths (n = 4) and deaths of unknown causes (n = 3), the overall 5-year survival rate was 63.3%. Mean overall survival time of the study population (n = 109) related to cancer-related causes was 8.8 ± 0.5 years. Median survival time could not be achieved, because <50% of the patients died within the follow-up period. In univariate survival analysis sex, age, tumor location, tumor size, presence of lymph node metastasis, and grading were of no prognostic significance in this study group. In addition, the variables related to surgical treatment, such as the type of resection used, the period of time between the end of radiotherapy and surgery, and the distance between the edge of the tumor and the margin of bowel resection, showed no significant difference in overall survival. In the clinicopathological variables, only TNM stage and the pT category were significantly associated with overall survival. When compared with stage I with II/III, the 5-year survival rate (65%
 versus 51.7%) differed significantly (P = 0.044) as well as pT_{1/2} compared with pT_{3/4} (5-year survival rate, 65% versus 50%; P = 0.05). The group of patients with a reduced or eliminated proportion of aneuploid cells in tumor [group AN (↓/0)], n = 56; DIDI, n = 23; AN (=↑), n = 26. P = 0.0004 (log-rank test).

The pretreatment aneuploid group had a nonsignificant tendency to improved survival (aneuploid: 5-year survival rate, 65.4%; diploid: 5-year survival rate, 55.1%). The reduction in SPF of diploid tumors was also associated with a tendency to improved survival (aneuploid: 5-year survival rate, 74%) versus patients with no decrease in the proportion of aneuploid cells [group AN (=↑)]; 5-yr 81.2%; 56.5%; 34.6%; P = 0.0004; Fig. 2]. The pretreatment aneuploid group had a nonsignificant tendency to improved survival (aneuploid: 5-year survival rate, 65.4%; diploid: 5-year survival rate, 55.1%). The reduction in SPF of diploid tumors was also associated with a tendency to a better prognosis (5-year survival rate, 72.6% versus 50%; P = 0.14), but the significance level could not be reached because of a small case number. The group of patients with tumors showing an increase of necrosis of >10% after radiotherapy had a more favorable prognosis (5-year survival rate, 74%) than patients with <10% increase (5-year survival rate, 63.9%) or patients with no increase of necrosis in the tumors (5-year survival rate, 46.4%; P = 0.003; Fig. 3 represents the related survival curves).

**Multivariate Cox Regression Analysis.** The only variables of independent prognostic significance by multivariate regression analysis were tumor TNM stage, DNA ploidy group, and increase of necrosis (Table 3).

**Table 3 Multivariate Cox regression analyses of clinicopathological and flow cytometric characteristics for overall survival in preoperatively irradiated rectal cancer**

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male vs. Female</th>
<th>NS*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>64.7 yr vs. &gt;64.7 yr</td>
<td>NS</td>
</tr>
<tr>
<td>TNM stage</td>
<td>Stage I vs. II/III</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>Histological grade</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Grade 1/2 vs. 3</td>
<td>0.056</td>
</tr>
<tr>
<td>Tumor location from anal verge</td>
<td>4 to &lt;7 cm vs. 7 to &lt;12 cm vs. ≥12 cm</td>
<td>0.048</td>
</tr>
<tr>
<td>Ploidy group</td>
<td>AN (↑) vs. AN (↓/0) vs. DIDI</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>AN (↑) vs. DIDI</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>Increase of necrosis</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>0% vs. &lt;10% vs. ≥10%</td>
<td>NS</td>
</tr>
</tbody>
</table>

*NS, not significant.

**DISCUSSION**

In this report, we found that changes in the ploidy status and cell proliferation, determined by flow cytometry, as a result of preoperative irradiation are of predictive value in the prognosis of patients with rectal cancer. This is the first study to compare ploidy status and cell proliferation before and after irradiation and to correlate the alterations with the clinical outcome of the patients. Previous investigations of DNA ploidy in colorectal cancer were mainly conducted in the operatively resected tumors without any previous neoadjuvant therapy. Thus, a direct comparison of these investigations only exists to our flow cytometric results of the pretreatment biopsy samples. Usage of stored tumor material offers the advantage that long-term follow-up is available. Other investigators have shown, in comparative studies of fresh and paraffin-embedded tumor tissue, that the determination of DNA ploidy is accurate and a reliable method (16, 17). In the present study, aneuploidy was observed in 86 of 116 preirradiated rectal adenocarcinomas (74%). This percentage is in accordance with most of the results of several other studies of rectal cancer (18–20). Slight differences in percentage of aneuploidy result from the fact that in Anglo-American studies the colon and rectal cancers were often evaluated together. This led to a decrease in the overall percentage of aneuploid tumors, which was also shown by Costa et al. (20) and Rognum et al. (21), who have observed that aneuploid tumors are more frequently represented in distal sections of the
large bowel. In accordance with other authors (22–24), the pretreatment nondiploid rectal carcinomas tended to cumulate around the triploid level with a mean DI of 1.67.

As described previously, we classified tumors as aneuploid only after evaluating all excised biopsies or paraffin blocks of these tumors if at least one showed a separate peak in the DNA histogram. In consideration of the known heterogeneity in solid carcinomas (25), Quirke et al. (26) reported that if only one excisional biopsy of a colorectal carcinoma was taken and analyzed by flow cytometry, the probability of determining aneuploid cells is 72% accurate. With an average count of 4.7 excisional biopsies/tumor before radiation and 2.5 paraffin blocks containing the resected tumor after irradiation, the chances to overlook aneuploid cells in our study are therefore minimal. The heterogeneity in solid carcinomas does not only influence the results of ploidy analyses but also the results of cell cycle compartment analyses (27). For that reason, the proportion of SPF in diploid colorectal cancers described in previous studies ranged from 5.8% to 11.7% (23, 27), compared with 7.4% in the present study. The mean SPF of pretreatment aneuploid carcinomas was calculated to be significantly higher (19.5%) than for diploid carcinomas, confirming the data of most other reports (22, 28).

Clinical studies of irradiated solid cancers showed that the percentage of aneuploid tumors was clearly reduced by irradiation (29–31). For example, in esophageal cancer, the rate of DNA aneuploidy was significantly reduced by irradiation (71% versus 47%; Ref. 9); however, this comparison was done in two separate groups of patients with and without preoperative irradiation. These results are confirmed in the present study, showing a reduction in aneuploidy from 74 to 48% after irradiation as an intraindividual comparison. The reduction in the number of aneuploid tumors presumably reflects that aneuploid cells undergo apoptotic cell death after DNA damage. Mohr et al. (31) reported that loss of aneuploidy was associated with an increase of necrosis in histological sections. Moreover, aneuploid carcinomas appear to be more radiosensitive, because in this study the increase of necrosis after irradiation was observed more frequently in aneuploid than in diploid tumors. In addition, survival of patients was significantly improved after induction of necrosis after irradiation.

The influence of the aneuploid DI in determining the likelihood of radiosensitivity is beyond dispute. Tetraploid bladder cancers are reported to have a greater responsiveness to irradiation with a more favorable clinical outcome (8, 32). This observation is in accordance to our results, showing that the percentage of tetraploid tumors was significantly higher in the group of aneuploid tumors with an eliminated aneuploidy. Furthermore, tetraploid tumors relapsed distally significant less frequently (5%) than near diploid (58%) or triploid tumors (37%). Therefore, it can be hypothesized that DNA tetraploidy might be an indicator for response to preoperative radiotherapy in rectal cancer. This assumption, however, still has to be supported by prospective studies.

Because radiation effects are seen mainly in tissues with a high rate of cell turnover (33), Kubouchi et al. (34) have noted that radiosensitivity in rectal cancer depends on the proliferative activity of cells. This study revealed a correlation between reduction in PCNA activity after radiotherapy in the patients with high initial levels of PCNA activity. This could be confirmed in the present study, in which the groups of diploid as well as aneuploid tumors with a reduced SPF after irradiation were found to have a significantly higher initial proportion of SPF. However, there was no significant influence on local or distant failure between the different percentage of S-phase in the pretreated diploid tumors, whereas a trend for improved survival was found in patients with diploid tumors, who had a reduced SPF after radiotherapy. Accordingly, in aneuploid tumors an initial SPF of <20.3 (median as cutoff; Table 2) was associated with a significantly higher recurrence rate and this led to a borderline significant lower 5-year disease-free survival rate (data not shown).

The apoptotic cell death after DNA damage to aneuploid tumor cells after irradiation results in an elimination or decrease of aneuploidy. The tumors with a reduced or eliminated proportion of aneuploid cells after radiation rarely relapsed locally (5.8%) compared with constant diploid tumors (13.6%) or tumors with an increased proportion of aneuploid tumor cells (27%). Furthermore, the clinical outcome was significantly different between these three defined ploidy groups. Therefore, 67% of the preirradiated aneuploid tumors [group (AN (1 / 4)/)] seem to have a higher radiosensitivity with a more favorable prognostic trend in contrast to the other 33% with an increased proportion of aneuploidy despite radiation. Several cell kinetic studies reported that response to radiation depends on multiple intrinsic and extrinsic factors (33). These factors, influencing radiosensitivity in a complicated still unknown way, might explain the differences in radioresponsiveness within the aneuploid tumors.

In this study, no significant difference in overall survival was observed for the tumor characteristic location and lymph node metastasis. This observation is in contrast to several studies (35, 36), defining tumor location and lymph node status as important prognostic factors in rectal cancers. Thus, preoperative radiotherapy seems to eliminate the prognostic meaning of these factors, which was also concluded by Sarashina et al. (37). The TNM stage and pT category were still of significant predictive value.

In conclusion, we propose that detection of alterations in the proportion of aneuploid cells after irradiation has a good prognostic value in preoperatively irradiated rectal cancer, and these results will help to distinguish between radiosensitive and radioresistant tumors. In addition, changes in the cell proliferation rate after radiotherapy might be helpful in detecting patients likely to benefit from preoperative radiotherapy. Further investigations of cell cycle kinetics during radiotherapy will be required to elucidate the precise mechanisms of tumor response to radiation and to optimize the individualized application of radiotherapy.

REFERENCES


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