Material and methods: Between 1993 and 2002, 183 patients with locally advanced rectal cancer (cT3/T4 or N+) were enrolled in this study. Preoperative chemoradiation consisted of 50.4 Gy of pelvic radiation with concurrent 5-fluorouracil+leucovorin bolus i.v. chemotherapy in 94 patients or oral capecitabine in 89 patients. Surgery was performed 6 weeks after chemoradiation. EGFR expression in the pretreatment paraffin-embedded tumor biopsy specimens was assessed by immunohistochemistry using an EGFR pharmDx kit (DakoCytomation). EGFR expression was determined from the intensity and extent of staining. The staining threshold for a positive result was 1+intensity in 1% of the tumor cells. EGFR immunostaining was graded as a categorical variable using an immunoreactive score (IRS) that ranged from 0 (negative staining) to 7 (strong staining) and was defined as low (IRS 0 to 3) or high (IRS 4 to 7) expression. Tumor downstaging was defined as a reduction in the pretreatment T stage by one level compared with the pathological stage. The predictive value of EGFR expression for tumor downstaging was evaluated using the chi-square test and logistic regression analysis.
Results: The median age of the patients was 59 years, and there were 111 males and 72 females. The preoperative clinical T stage was T3 in 163 patients (89%) and T4 in 20 patients (11%). Tumor downstaging occurred in 97 patients (53%). The tumor showed a pathologic complete response in 27 patients (15%). Positive EGFR expression was observed in 140 of 183 patients (76%). The grade of EGFR expression was low in 113 patients (62%) and high in 70 patients (38%). High EGFR expression was not correlated with gender, age, tumor mobility, tumor size, tumor distance from the anal verge, cT stage, cN stage, or pN stage, but was correlated with pT stage ($p = 0.043$). EGFR expression and age were marginally significant predictive factors for tumor downstaging in the univariate analysis ($p = 0.063$ and 0.081, respectively). In the logistic regression analysis, including the variables EGFR expression, age, tumor mobility, and cN stage, high EGFR expression was the only significant predictive factor for tumor downstaging (hazard ratio 0.515, 95% confidence interval 0.276 to 0.963, $p = 0.038$).
Conclusions: High EGFR expression is a significant predictive molecular marker for tumor downstaging in locally advanced rectal cancer treated with preoperative chemoradiation.

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Prevalence of high-risk lesions in prophylactic mastectomy specimens of 82 BRCA1 and BRCA2 mutation carriers

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Purpose: Women with a hereditary predisposition for breast cancer have a very high risk (up to 85%) of developing invasive breast carcinoma and consider prophylactic mastectomy to avoid this risk. Together with cancer-free survival, the effectiveness of prophylactic mastectomy in *BRCA1* and *BRCA2* carriers may be established by the spectrum of high-risk lesions in their mastectomy specimens. Little is known about differences between early stages of breast cancer development in *BRCA1* and *BRCA2* mutation carriers. It is unknown whether the prevalence of high-risk lesions in *BRCA1* and *BRCA2* mutation carriers is different. There may be differences in breast cancer development in *BRCA1* and *BRCA2* mutation carriers because the features of invasive breast cancer lesions are different.
Patients and methods: A prospective series of 68 *BRCA1*- and 14 *BRCA2*-prophylactic mastectomy specimens was analyzed by radiography and macroscopic inspection of 5 mm tissue slices and histological examination of suspicious lesions and random samples from each quadrant of the breast and the nipple area.
Results: Patient characteristics of the two groups were comparable for age at time of prophylactic mastectomy (36 $\pm$ 9 years), presence of previous breast cancer (35%), age at previous breast cancer (42$\pm$9 years), postmenopausal status (46%) and previous oophorectomy (23%). The earliest age of breast cancer occurrence was significantly younger (35 $\pm$ 9 years) in *BRCA1* than *BRCA2* families (44$\pm$9 years; $p = 0.02$). High-risk lesions are equally frequent among women with a *BRCA1* or a *BRCA2* mutation: all high-risk lesions 44% versus 36% ($p = 0.56$), atypical lobular hyperplasia 26% versus 21% ($p = 0.69$), atypical ductal hyperplasia 18% versus 14% ($p = 0.70$), lobular carcinoma-in-situ (LCIS) 16% versus 7% ($p = 0.38$) and ductal carcinoma-in-situ (DCIS) 9% versus 7% ($p = 0.83$).
Conclusions: The high prevalence of high-risk lesions associated with an increased risk of malignancy, substantiates the generalized nature of incipient malignant changes both in *BRCA1* and *BRCA2* mutation carriers and confirms the indication for prophylactic mastectomy. Surveillance does not detect these high-risk lesions.

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Cytoplasmic p27 kip-1 expression is an indicator of good prognosis in colorectal cancer patients

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Introduction: The p27kip-1 protein inhibits certain cyclin-CDK complexes in the cell nucleus, thereby preventing uncontrolled cellular proliferation. Recent data suggests that cytoplasmic p27kip1 may have an alternative function, inhibiting the activity of cytoplasmic Rho proteins which co-ordinate cytoskeletal remodelling and underlie changes in cell adhesion and migration. Our aim was to evaluate the prognostic significance of cytoplasmic and nuclear p27kip-1 in a large series of colorectal cancer patients.
Methods: Using high-throughput Tissue microarray (TMA) technology, we analysed p27kip-1 cytoplasmic and nuclear expression in a series of over 400 paraffin embedded colorectal tumor specimens. Data derived from this analysis was associated with known patient and tumor variables, and with long-term patient outcome data, in order to gain further insight into the mechanisms by which p27kip-1 may influence tumor development.
Results: 74/418 tumours expressed both cytoplamsic and nuclear p27kip-1 which was not associated with the known clinicopathological variables including tumor stage, tumor grade or the presence of vascular invasion. However, on survival analysis using the Kaplan-Meier method there was a significant correlation between p27kip-1 expression and disease specific survival ($p = 0.037$), with patients whose tumours express both nuclear and cytoplamsic p27kip1 having a good prognosis. In contrast, expression of nuclear p27kip-1 alone was observed in 217/418 (51.9%) tumours, and this did not demonstrate any correlations with clinicopathological variables or survival.
Conclusions: For tumours to metastases, cells must alter their connections to their neighbours and their substrate, and then migrate. Efficient migration requires a tight balance between activation and deactivation of Cdc42, Rac and RhoA in both time and space. Sequestration of RhoA by cytoplasmic p27kip-1 may inhibit migration by preventing cells from achieving sufficiently strong adhesion and traction to move forward. In this study cytoplasmic expression of p27kip-1 was always associated with nuclear expression. These tumours would therefore have controlled proliferation and reduced migration resulting in a less aggressive tumour and a good prognosis. These finds support recent evidence that cytoplamsic p27kip1 expression has an important biological role that can influence tumour outcome.

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Bromodeoxyuridine labelling index as an indicator of tumour response to neoajuvant radiotherapy in patients with rectal cancer

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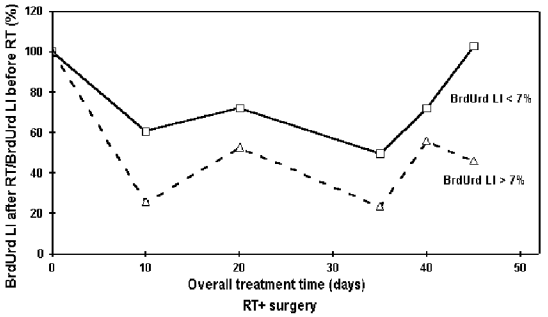
Background: In clinical practice there are no certain methods to predict tumour response to neoadjuvant radiotherapy (RT). Therefore the aim of the study is an assessment of tumour proliferation rate based on Bromodeoxyuridine labelling index (BrdUrd LI) to predict tumour response to neoadjuvant RT in patients with rectal cancer.
Material and methods: Tumour samples were taken twice from each of 65 patients with rectal carcinoma qualified to neoadjuvant RT: before RT and during surgery. Tumour fragments were incubated with BrdUrd for 1 hour at 37°C, and after fixation and staining the cell preparations were analysed with flow cytometer. The BrdUrd LI was calculated as a percentage of BrdUrd-labelled cells in a sample which incorporated BrdUrd. S-phase fraction (SPF), DNA ploidy, and apoptosis were also evaluated. Patients were treated according to two RT schedules: I, short RT for 5 days with 5 Gy/fraction and surgery about one week after RT, or II schedule: short RT (5×5 Gy) with longer interval, 4–5 weeks before surgery. Tumour response after RT has been evaluated by a patologist on the basis of tumour material taken during surgery.
Results: Thirty-one patients were treated according to schedule I, in which the mean interval before surgery was 8 days (range 2–14). In 34 patients schedule II was applied, in which mean break was 32 days (range 17–45). Mean BrdUrd LI before RT was 7% (range 1.0–24.2%) and the mean value did not differ between the two schedules. After RT, tumours treated according to both schedules showed statistically significant growth inhibition (reduction of BrdUrdLI and percentage of SPF cells) in comparison with the values obtained before RT. Because the interval

between RT and surgery appeared to be longer than planned, overall treatment time (OTT), e.g. time from the beginning of RT to surgery was calculated and it was found to be 7–50 days. Radiation induced inhibition of tumour proliferation was expressed as a percentage of the BrdUrd LI obtained after RT/BrdUrdLI obtained before RT. This ratio was calculated separately for faster (BrdUrd LI >7%) and slowly (BrdUrd LI ⩽7%) proliferating tumours and correlated with OTT. The figure shows significant reduction of the mean BrdUrd LI after RT in faster proliferating tumours (27 patients), and smaller inhibition of tumour proliferation in slowly proliferating tumours (38 patients), even for long OTT.



Tumour response to neoadjuvant RT

Conclusion: On the basis of BrdUrd LI it is possible to predict tumour response after neoadjuvant RT. However, clinical usefullness of this method should be confirmed by finding the correlation between BrdUrdLI and the results of treatment.

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New intraoperative molecular diagnosis for lymph node metastasis in breast cancer

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Background: The sentinel lymph node (SLN) biopsy has been becoming standard procedures for early stage breast cancer patients. Intraoperative frozen section of SLN can be used for detection of metastasis; however, it is considered that intraoperative diagnosis would not be high sensitivity and increase work-load in the pathology laboratory. The molecular diagnosis has shown more sensitive than conventional method, but it needs several hours for analysis. To address these problems, we establish a new intraoperative molecular diagnosis based on one-step nucleic acid amplification (OSNA) with a quantitative measurement of cytokeratin 19 (CK19) mRNA.
Methods: A quantitative OSNA assay was developed to measure cytokeratin 19 mRNA expressions, which consists of the sample preparation step and the rapid gene amplification by RT-LAMP (reverse-transcriptase loop-mediated isothermal amplification). All processes of OSNA assay are easy operation and it takes within 30 min to accomplish analysis. Retrospective study; Frozen 106 LNs of 36 patients were analyzed CK19 mRNA expression for the determination of cutoff value. Prospective study; Fresh 116 LNs of 36 patients including 48 SLNs of 30 patients were analyzed for the evaluation of the intraoperative performance of OSNA assay during surgery in Osaka Police Hospital.
Result: Retrospective study; CK19 mRNA expressions of histological negative LNs were less than 3×10^2 copy/reaction. From this results, it was determined the cutoff value of CK19 mRNA copy number as 500 copy/reaction in CK19 OSNA assay. By using this cutoff value, there was a 98.1% (104/106) concordance between the CK19 OSNA assay and histopathological diagnosis at 2.0 mm intervals; the sensitivity was 100% (22/22), and the specificity was 97.6% (82/84). Prospective study; the concordance of CK19 OSNA assay was 96.6% (112/116). The specificity and sensitivity were 92.3% (24/26) and 97.8% (88/90), respectively. Two case of false negative may be caused by difference of sampling reign, because these LNs contained only micrometastases.
Conclusions: Our results showed that CK19 transcription was an excellent molecular marker for LN metastasis diagnosis. In addition, the rapid method, CK19 OSNA assay, is applicable to intraoperative diagnosis for the sentinel node biopsy in breast cancer. This is the first report of intraoperative molecular diagnosis of lymph node metastases in breast cancer.

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A novel high through-put screening for transcription factor target genes

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The GATA family transcriptionally regulates differentiation of hematopoietic cells. In particular, GATA3 has been demonstrates to accomplish an important role in differentiation of epithelial cells.
In this study, we developed a novel approach identifying transcription factor-binding genes, termed sChIP. In order to delineate the GATA3-target genes in HaCaT keratinocytes, we performed sChIP, and obtained 173 clones and 134 different fragments that mapped unique genomic loci; 35 were mapped within 100 kbp upstream from the transcription start site, 18 were upstream of 100 kbp, 49 were located at near 3'ends of annotated genes, and the rest of 32 were intragenic regions. We categorized them according to molecular activities; transcription and nuclear factor; 20, signaling molecule; 16, metabolic molecule; 31, cell-cell adhesion and extracellar matrix; 16, others; 13, unknown function; 38. Transcriptional activation of the target genes was exemplified by RT-PCR.
In the present study, we developed sChIP, which effectively identifies the target genes and prospects functionalities of transcription factors.

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Pharmacogenetic study in patients (pts) with metastatic breast (MBC) and colorectal cancer (MCRC) treated with capecitabine (C)

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Background: Carboxylesterase (CES) and cytidine deaminase (CDD) are involved in hepatic transformation of C to 5'dFUrd. Thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) polymorphisms are known to correlate with the efficacy and toxicity of 5-FU. Different polymorphisms of these enzymes could also be responsible for variations in efficacy and toxicity in different pts receiving C. Therefore, we evaluated pts with previously treated MBC and MCRC to determine if there were any correlations between genetic polymorphisms of CES, CDD, TS and DPD and efficacy and/or grade 3/4 toxicity of C.
Methods: The study included pts with evaluable or measurable MBC or MCRC, no dermatological disease and normal hepatic function. All pts received standard C 1250 mg/m^2 orally bid d1–14 every 3 weeks until progressive disease or intolerable toxicity. PCR and sequencing methods were used to analyse the following: CES exon 3 (5841 G > A, 6046 G > A, 6174 G > A, 6320 G > A); CES UTR (823 C > G, 854 G > C); CDD (575 C > T, 771 C > G, 794 G > A, 942 C > G, 943 insC, 1052 A > C); TS genotype (2R/2R, 2R/3R, 3R/3R); and DPD (IVS14+1 G > A). Fisher's exact test was used for comparisons.
Results: Baseline characteristics of the 109 enrolled pts (58 MBC/51 MCRC) were: median age 62 yrs (32–90); ECOG PS (88% 0–1; 12% ⩾ 2); gender (26% male, 74% female). Most pts (70%) had received prior treatment for metastatic disease, with 34% of pts having received ⩾2 prior treatments. Almost half of the pts (49.5%) had hepatic metastases. The most common grade 3/4 adverse events were: hand–foot syndrome (HFS, 18%), asthenia (6%), diarrhoea (5%), mucositis (3%) and nausea/vomiting (3%). A significant correlation was detected between heterozygous (HT) and homozygous (HM) polymorphisms of the CDD gene 943 insC and an increased rate of grade 3 HFS compared with the wild type (WT) (27%, 13% and 6%, respectively, p = 0.03). 87 pts were evaluable for response (CR 5%; PR 39%; SD 31%). 71% of pts with HT and 50% with HM polymorphisms of the 5′ Untranslated Region of the CES 2 gene (CES 2 5′UTR 823 C/G) had a CR or PR compared with 35% of pts with the WT (p = 0.007). In the MBC group, 66% of pts with the 2R/2R variant of TS and 42% with 2R/3R had CR or PR compared with 0% of those with a 3R/3R form (p = 0.02). Multivariate Cox regression analysis showed that the response rate correlated with the presence of hepatic metastases (p = 0.0015) and the CES 2 5′UTR (823 C/G) polymorphism (p = 0.0024).
Conclusions: A 943 insC polymorphism in the CDD gene appears to be associated with a higher rate of grade 3 HFS. The occurrence of an 823 C/G switch at the CES 2 5′UTR gene may be associated with higher efficacy of C. These findings need to be confirmed in larger samples and the impact of these polymorphisms on the amount and activity of the encoded proteins needs further investigation. The study is ongoing.