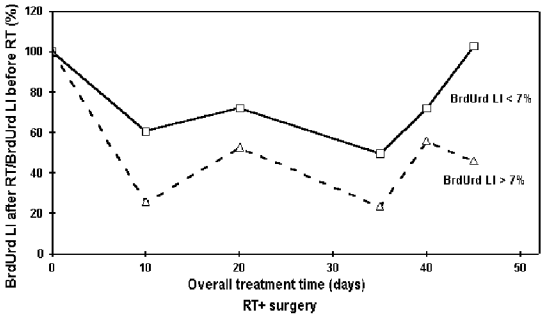
between RT and surgery appeared to be longer than planned, overall treatment time (OTT), e.g. time from the beginning of RT to surgery was calculated and it was found to be 7–50 days. Radiation induced inhibition of tumour proliferation was expressed as a percentage of the BrdUrd LI obtained after RT/BrdUrdLI 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.