1317 POSTER

The comprehensive analysis of UGT1A genetic polymorphisms in patients with metastatic gastrointestinal cancer treated with irinotecan chemotherapy

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Background: Uridine diphosphate-glucuronosyltransferases (*UGT*)*1A1*28* and *UGT1A1*6* have been reported to associate with irinotecan (CPT-11)-induced neutropenia. We analyzed the association among *UGT1A* genetic polymorphisms comprehensively in patients (pts) with metastatic gastrointestinal cancer enrolled in the UGT0601 genotype-directed dose finding study.
Material and Methods: Pts received prior chemotherapies except for CPT-11 for metastatic gastrointestinal cancer were enrolled. CPT-11 was administered biweekly. PK sampling was evaluated during the first cycle. The polymorphisms of *UGT1A1*28* and *UGT1A1*6* was detected by Invader Assay®. Those of *UGT1A7* (*387T>G*, *391C>A*, *392G>A*, *622T>C*), and *UGT1A9*22* were detected by direct PCR sequencing kit BigDye®. Those of *UGT1A1*27*, and **60* were detected by TaqMan® SNP Genotyping. We examined the association of *UGT1A* genotypes with adverse events (AE), and PK profile. This study is registered with UMIN Clinical Trial Registry, number UMIN000000618 and supported by Yakult Honsha Co., Ltd.
Results: Of 82 pts enrolled, 76 provided informed consent for this analysis. Allele frequency of *UGT1A1*28*, *UGT1A1*6*, *UGT1A9*22*, *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), and *UGT1A7* (*622T>C*) were 0.118, 0.237, 0.368, 0.388, 0.289, respectively. UGT1A7 (*387T>G*, *391C>A*, *392G>A*) was highly linked with *UGT1A9* ($r^2 = 0.92$). We also found the linkage association between *UGT1A7* (*387T>G*, *391C>A*, *392G>A*) and *UGT1A7* (*622T>C*), and *UGT1A7* (*622T>C*) and *UGT1A1*6* (r^2=0.64, 0.59, respectively). In the AE analysis, the incidence of grade 3 or 4 hematological toxicity was higher in pts with *UGT1A1*28*, *UGT1A1*6*, *UGT1A9*22*, *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), and *UGT1A7* (*622T>C*) allele (neutropenia: P = 0.01, P = 0.01, P = 0.007, P = 0.002, P = 0.001; respectively). Compared the wild-type and heterozygote with homozygote, the incidence of grade 1 or more anorexia was higher in pts with *UGT1A1*6*, *UGT1A9*22*, *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), and *UGT1A7* (*622T>C*) (P = 0.028, P = 0.046, P = 0.012, P = 0.001, respectively). In the PK analysis, pts with *UGT1A1*28*, *UGT1A1*6*, *UGT1A9*22* and *UGT1A7* (*387T>G*, *391C>A*, *392G>A*) allele showed the similar trend for SN-38 $AUC_{0-24\,h}$ (P = 0.012, P = 0.012, P = 0.018, P = 0.005; respectively).
Conclusions: Our results indicate that *UGT1A7* (*387T>G*, *391C>A*, *392G>A*), *UGT1A7* (*622T>C*) and *UGT1A9*22* may also be predictive marker for safety in CPT-11 therapy.

1318 POSTER

A new duplex real-time PCR assay for detection of the mSEPT9 biomarker for colorectal cancer screening using blood plasma

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Background: Despite the good prognosis for colorectal cancer patients when disease is detected early, compliance to screening programs remains low. Concomitants and inconvenience of available methods point to the need of convenient and reliable tests with the potential to increase patient adherence. Previously we have shown in 3,000 patient plasma samples that the detection of methylated DNA of the SEPT9 gene (mSEPT9) is strongly associated with the presence of colorectal cancer. Here, we present data generated with a new workflow that utilizes a duplex real-time greatly simplifying reliable mSEPT9 detection in human plasma.
Material and Methods: DNA from human plasma is extracted, bisulfite converted, and finally purified with the workflow developed by Epigenomics. The output DNA is suited for detection via real-time PCR. Detection of DNA is accomplished via a duplex PCR combining a highly sensitive methylation specific SEPT9 DNA detection assay with a beta-actin assay used as an internal control. The entire workflow has been optimized to increase robustness and improve the ease-of use. Comparative data were generated on technical samples prepared from human plasma and spiked with concentrations of methylated DNA (mDNA). Plasma aliquots with a dilution series of mDNA spikes were measured repeatedly. In addition, 100 sample aliquots derived from clinical patient material were measured. The results were compared to data generated by the published reference method (mSEPT9 Detection Assay, Epigenomics).
Results: Both workflows consistently detected a mSEPT9 signal ($\geqslant$95% of replicates) in aliquots with a 30 pg/ml spike of mDNA. The observed standard deviation for repeated measurements was slightly lower for the new duplex workflow when compared to the reference method. For the clinical samples we observed excellent agreement of results between the two methods in more than 85% of the valid cases.
Conclusions: The biomarker mSEPT9 has been established in several independent studies to be ~70% sensitive to detect colorectal cancer at ~90% specificity. The newly developed duplex workflow for mSEPT9 detection in human plasma shows excellent agreement with, and therefore is considered substantially equivalent to, the reference method. This new assay, which has been demonstrated to accurately detect mSEPT9 in a standard blood specimen, is expected to significantly improve patient. The design and robustness of the assay will enable its use in standard routine laboratory procedures.

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Microsatellite instability in sporadic colorectal cancer: correlation with novel clinical parameters

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Background: Microsatellite instability (MSI), present in up to 15% of sporadic colorectal cancers (CRC), is associated with clinico-pathologic features including right sided cancers and increased age. Associations with other clinical parameters have yet to be fully explored.
Methods: MSI, using Bethesda 5-panel markers, was evaluated in primary CRC tumors. Samples were selected to provide an even spread of tumor location (right colon, left colon and rectum) and patient age. Clinico-pathologic features of patients with microsatellite stable (MSS) versus MSI tumours were compared using a prospectively collected clinical database.

Table 1: Clinico-pathologic parameters, MSI versus MSS tumors

	MSI-H (n = 78)	MSS (n = 493)	P value
Median age	76.5	69	0.001
Site of tumor	n (%)	n (%)	
Right colon	57 (73.08)	163 (33.06)	<0.0001
Left colon	12 (15.38)	167 (33.87)	
Rectum	9 (11.54)	163 (33.06)	
Stage at presentation	N = 78	N = 492	
A	4 (5.13)	41 (8.33)	Stage D vs other = 0.0009
B	21 (26.92)	91 (18.5)	
C	46 (58.97)	231 (46.95)	
D	7 (8.97)	129 (26.22)	
Median lymph node yield	N = 78	N = 485	0.007
	17.5	14	
BMI (median, kg/m^2)	N = 43	N = 279	
	28.3	26.6	0.07
Grade of differentiation	(n = 77)	(n = 485)	
well	0 (0)	4 (0.82)	
moderate	31 (40.26)	300 (61.86)	
poor	41 (53.25)	174 (35.88)	Poor vs other = 0.0009
not reported	5 (6.49)	7 (1.44)	
Gender			
Male	38 (48.05)	249 (50.51)	0.71
Female	40 (51.95)	244 (49.49)	
Smoking status	(n = 77)	(n = 485)	
current	10 (12.99)	61 (12.58)	0.87
ex	22 (28.57)	127 (26.19)	
non	45 (58.44)	297 (61.24)	
Type I/II diabetes	(n = 77)	(n = 488)	
yes	19 (24.68)	103 (21.11)	0.46
no	58 (75.32)	385 (78.89)	

Results: Tumors from 571 patients were evaluated. Results are presented in Table 1. In total 78 tumors (13.7%) were MSI and 493 (86.3%) were MSS. The majority of MSI tumors occurred in older patients, were right sided, and poorly differentiated. Patients with MSI were significantly less likely to

present with Stage D disease (9% versus 26%, p = 0.0009). The median lymph node yield at surgery was significantly higher for MSI CRC (17.5 versus 14 nodes, p = 0.007). There was trend for a higher median body mass index (BMI) in patients with MSI CRC (28.3kg/m^2 versus 26.6kg/m^2, p = 0.07). Neither smoking nor diabetes were associated with MSI.
Conclusions: In this selected patient cohort we found a higher lymph node yield in association with MSI, and a possible association with increased BMI. We could not confirm the previously reported association between smoking and MSI. These findings should be explored further as they may provide insight into the biology underlying the development of MSI CRC.

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Potential of heat shock protein 90 expression in the nucleus as a useful parameter for neoadjuvant chemotherapy in gastric cancer

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Purpose: Heat shock protein 90 (Hsp90) is a molecular chaperone that plays crucial roles in cellular responses to stressful conditions. Most studies related to cancer treatment have focused on cytoplasmic Hsp90. However, Hsp90 is also found in the nucleus, albeit at considerably smaller levels (Dote et al. Cancer Res. 2006;66:9211−20). Our previous study showed that Hsp90-negative expression correlated with more aggressive disease and could provide useful prognostic information for gastric cancer patients (Dote et al. AACR2009 abstract #1632). Neoadjuvant chemotherapy with S-1/cisplatin has shown some success in the treatment of gastric carcinoma, but objective parameters for measuring its effects are lacking. In this study, we studied the correlation between Hsp90 expression and the histological chemotherapeutic effect in advanced gastric cancer with S-1/cisplatin neoadjuvant chemotherapy.
Material and Methods: Sixteen primary advanced gastric cancer patients were recruited into the study. Two cycles of continuous oral administration of S-1 (100–120 mg/body/day, 21 days) plus drip infusion of cisplatin (60 mg/m^2/day, Day 8) was performed as neoadjuvant chemotherapy. Histological chemotherapeutic responses of the resected specimens were classified into good responders and poor responders. Hsp90 expression on formalin-fixed paraffin-embedded specimens both before and after neoadjuvant chemotherapy was examined immunohistochemically. Chi-square test and Kaplan-Meier analysis were used for statistical analysis.
Results: High expression level of Hsp90 in cytoplasm (defined as stronger staining compared with adjacent normal gastric mucosa) was found in 7 tumors (44%) in pretreatment biopsy and 8 tumors (50%) in surgically resected specimens. There was no significant correlation between Hsp90 expression and pathological response and survival rates in both biopsy and surgical specimens. Interestingly, in 6 patients with recurrence, Hsp90 expression in the nuclei was observed in 3 surgically resected specimens. However, no significant difference was detectable because of the small number of patients.
Conclusions: Our results suggest that Hsp90 expression in the cytoplasm did not correlated with neoadjuvant chemotherapeutic effect and prognosis for advanced gastric cancer patients. However, the potential of Hsp90 expression in the nuclei as prognostic biomarkers for neoadjuvant chemotherapy warrants further validation.

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A novel epigenetic biomarker panel for early detection of colorectal cancer and adenomas

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Background: Diagnosis of colorectal tumors at an early resectable stage will significantly reduce colorectal cancer mortality. The presence of cancer-specific DNA methylation in epithelial cells shed into the lumen as well as in tumor-derived free DNA in blood serum makes a non-invasive approach to early detection of cancer possible. Biomarkers used in diagnostics or screening must have an optimal sensitivity and specificity. The purpose of the present study was to investigate a panel of novel epigenetic markers for the detection of CRC and adenomas.
Material and Methods: We used methylation-specific polymerase chain reaction to investigate the promoter methylation status of 14 previously identified candidates in colon cancer cell lines (n = 20). Seven were hypermethylated in >80% and were subjected to quantitative methylation analysis in test sets of CRC, adenomas, and normal mucosa. Findings were verified in validation series.
Results: Five of the candidates, *CNRIP1*, *FBN1*, *INA*, *SNCA*, and *SPG20,* harbored frequent promoter hypermethylation in colorectal carcinomas (66−94%) as well as in adenomas (43−92%). In contrast, methylation was rare among normal mucosa samples (0−7%). By combining all five genes in a biomarker panel and require two or more methylation positives, 93% of the colorectal carcinomas and 87% of the adenomas could be detected, with a specificity of 98%. Both benign and malignant tumors could be detected independent of clinical characteristics, such as tumor stage, location in the colon, microsatellite instability and *BRAF* mutation status, as well as the gender and age of the patient.
Conclusions: The novel epigenetic marker panel identified here demonstrates high and diagnostically promising sensitivity and specificity measurements for colorectal carcinomas as well as adenomas. The findings underline that this biomarker panel will be highly suitable for early detection of colorectal cancer and adenomas.

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Diagnostic of KRAS gene mutations in colorectal cancer: evaluation of direct sequencing, pyrosequencing and allele specific amplification

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Background: Mutations of the KRAS gene are are predictive of response to anti-EGFR drugs in colorectal cancer. Direct sequencing could be considered as the gold standard to determine single base substitution. Recently, different rapid techniques showing high sensitivity have been developed. The aim of this study is to compare different KRAS mutation detection methods suitable for use in clinical routine.
Materials and Methods: DNA extracted from cell lines with wild type (LNCaP) or mutated KRAS codon 12 sequence (SW620) and from 5 µm paraffin embedded sections of colo-rectal tumors was used to perform detection of KRAS mutations. Three different methods were compared.
(1) Direct sequencing of amplification products were performed in both the forward and reverse directions using automated fluorescence dideoxy sequencing (ABI 3130 genetic analyser). (2) Pyrosequencing was performed by using the KRAS v2.0 assay on the PyroMarkTM Q24 system (Qiagen). After PCR amplication of a DNA segment spanning codons 12 and 13, the "sequencing by synthesis" methodology quantifies mutations in these codons. (3) TheraScreen® KRAS Mutation was the first diagnostic test to obtain CE Mark certification for the detection of KRAS mutations in colorectal cancer. The test use real-time PCR and combine allele specific PCR (Amplification Refractory Mutation System®) with the Scorpions® technology to detect the 7 most common mutations founded in colorectal cancer
Results: Both methods, sequencing and pyrosequencing, were able to detect up to 5% of mutated alleles present in wild type genomic DNA. With the Therascreen test the c.35G>T, p.G12V mutation was reproducibly and unambiguously detected even when the mutated DNA represented 1% of the total DNA in reaction. The concordance rate between direct sequencing and pyrosequencing (n = 89 patients, 57 wild-type and 32 mutated) was 100%. The concordance rate between sequencing and Therascreen (n = 34 patients) was 85%. The discrepancies (n = 5 patients) were due to mutations detected by Therascreen but not by direct sequencing
Conclusions: The three methods used are able to detect mutations present at a rate of, at least, 5% in the sample. Direct sequencing allows an exhaustive detection of the different type of mutations. Pyrosequencing can be used to perform quantitative detection of the mutations. Therascreen test shows the highest sensitivity.

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mTOR expression in gastrointestinal (GI) tract poorly differentiated endocrine carcinoma (PDEC)

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Background: Neuroendocrine tumours (NETs) arise from the cells of the disseminated neuroendocrine system, which is widely distributed in the body. These are a relatively rare and heterogeneous group of neoplasms characterized by differences in embryologic, biologic, histopathologic aspects and also in their aggressiveness and prognosis. In particular GI tract PDEC are rare tumours accounting 0.1%-1% of all GI malignancies. mTOR signalling pathway has emerged as a promising target for well-differentiated endocrine carcinoma therapy. Because the biologic behaviour