

Prevalence of topoisomerase I genetic mutations and UGT1A1 polymorphisms associated with irinotecan in individuals of Asian descent

TOMOYA FUKUI¹, HISASHI MITSUFUJI¹, MASARU KUBOTA¹, HIDENORI INAOKA³, MINORU HIROSE³, KEIICHI IWABUCHI², NORIYUKI MASUDA¹ and HIROSUKE KOBAYASHI³

Departments of ¹Respiratory Medicine and ²Pathology, School of Medicine;
³Department of Medical Engineering and Technology, School of Allied Health Sciences,
Kitasato University, Kanagawa 252-0373, Japan

Received January 27, 2011; Accepted June 17, 2011

DOI: 10.3892/ol.2011.346

Abstract. Topoisomerase I (TOP-I) mutations have been shown to be correlated to irinotecan resistance *in vitro*. However, the prevalence of TOP-I germline mutations has yet to be systematically elucidated. On the other hand, polymorphisms of *UGT1A1* have been shown to be associated with CPT-11 toxicity in clinical situations. The primary aim of this study was to investigate the prevalence of mutations in the TOP-I exons associated with CPT-11 resistance, including untreated cancer tissue. A secondary aim was to confirm the less frequent *UGT1A1**28 and more frequent *UGT1A1**6 in individuals of Asian descent compared to Caucasians and individuals of African descent. The prevalence of 5 reported TOP-I mutations in exons was investigated in volunteers (n=236) using DNA sequencing of the PCR products. The prevalence of TOP-I mutations in untreated lung cancer tissues (n=16) was also investigated. Additionally, 3 *UGT1A1* polymorphisms, *UGT1A1**6, *27 and *28, were investigated in volunteers (n=126). There were no mutations of TOP-I in any of the 236 subjects or in the untreated lung tissues. Among 128 subjects, the distribution of homozygous polymorphisms of *UGT1A1* was: *UGT1A1**28 in 3 (2.4%) and *UGT1A1**6 in 4 (3.2%) subjects, and co-occurrence of heterozygous polymorphisms for both *UGT1A1**6 and *UGT1A1**28 in 4 (3.2%) subjects, and for *UGT1A1**27 and *UGT1A1**28 in 1 subject (0.8%). The Hardy-Weinberg deviation test showed there was no significant deviation from the equilibrium, and the association analysis indicated no significant linkage between *UGT1A1**6 and *UGT1A1**28. In conclusion, TOP-I genetic

mutations correlated to CPT-11 resistance were not detected in any of the subjects and untreated lung cancer tissues. Less frequent *UGT1A1**28 and more frequent *UGT1A1**6 were confirmed in East Asian individuals compared to Caucasians and individuals of African descent. Linkage disequilibrium was not detected between *UGT1A1**6 and *UGT1A1**28.

Introduction

Irinotecan hydrochloride (CPT-11), a water-soluble semi-synthetic derivative of camptothecin, has been shown to exert marked antitumor activity (1). It is an inactive prodrug, and its major metabolite SN-38 is a potent topoisomerase I (TOP-I) inhibitor. SN-38 stabilizes covalent TOP-I-DNA complexes, causing DNA strand breaks. Several point mutations of TOP-I were identified as being associated with resistance to CPT-11 (2-4), and structural models were introduced to explain how the mutations of TOP-I hinder the docking of camptothecin derivatives in the ternary complex of TOP-I-DNA (5-7).

The cultured cells with the TOP-I mutation showed no obvious or only minor defects in cell function and proliferation (2-4). Consequently, such mutations may be innocent or not fatal to the cells. Therefore, it is reasonable to consider that the mutations may even occur in germlines.

CPT-11 exhibits inter-individual variations in terms of both pharmacokinetic and pharmacodynamic behavior (8). CPT-11 is hydrolyzed to yield active SN-38 (9) and detoxified via glucuronidation of SN-38 by uridine diphosphate glucuronosyltransferase (UGTs) to yield its β -glucuronides, SN-38G (10). *UGT1A1* is the main isoform of UGTs involved in the formation of SN-38G. Genetic polymorphisms of the *UGT1A1* gene were revealed to explain the variability of CPT-11-related toxicity among patients, particularly *UGT1A1**28 [(TA)₇TAA], the existence of which is known to be predictive of CPT-11-induced neutropenia (11). In addition, the *UGT1A1**6 and *27 alleles, two variants in exon 1 of the *UGT1A1* gene, are found mainly among individuals of Asian descent, and have also been indicated to affect enzyme function (12,13). The associated phenotype of *UGT1A1**28, *UGT1A1**6, or *UGT1A1**27 is the Gilbert syndrome (14), and a strong association between

Correspondence to: Dr Hirosuke Kobayashi, Department of Medical Engineering and Technology, School of Allied Health Sciences, 1-15-1 Kitasato, Sagami-hara, Minami-ku, Kanagawa 252-0373, Japan
E-mail: hiro@kitasato-u.ac.jp

Key words: topoisomerase I, mutation, UGT1A1, polymorphism, CPT-11

A Protein sequence

MSGDHLHNSQIEADFRNLNDSHKHKDKHKDRHRHKEHKKEKDREKSKHSNSEHKDSEKHKH
 EKEKTKHKDGSSEKHKDKHKDRDKEKRKEEKVRA SGD AKIK KEKENG FSSPPQIKDEPEDDGYF
 VPPKEDIKPLKRPRDEDDADYKPKKIKTEDTKKEKKRKLEEEEDGKLKKPKNKDKDKK VPEPDN
 KKKKPKKEEQKWKW WEEERY PEGIKWK FLEHKGPFVAPPYEPLPENVKFYDYGKVMKLSPK
 AEEVATFFAKMLDHEYTTKEIFRKNFFKDWRKEMTNEEKNIITNLSKCDFTQMSQYFKAQTEAR
 KQMSKEEK LKIKEEN EKLLKEYGFCIMDNHKERIANFKIEPPGL ERGRGNHPKM²⁻¹ GMLKRRIMP
 EDIINCSKDAKVSPPPGHKWKEVRHDNKVTWLVSWTENIQGSIKYMILNPSSRIKGEKD WQKY
 ETARRLK KCV DKIRNQYREDWKS KEMKVRQRAVALYFIDKLALRAGNEKEEGETADTVG⁴CCSL
 RVEHINLHPELDGQEYVVEFDLGL KDS²⁻² IRYYNKVPVEKRVFKNLQLFMENKQPEDDLFDRLNT
 GILNHLQDLMEGLTAKVFRTYNASITLQQQLKELTAPDENIPAKILSYNRANRAVAILCNHQRAP
 PKTFEKSMNLQTKIDAKKEQLADARRDLKSAKADAKVMKDAKTKKVVESK KAVQRLEEQL
 MKLEVQATDREENKQIAL GTSKLN¹ YLDPRIT³ VAWCKKWGPVIEKIYNKTQREKFAWAIDMADE
 DYEYF

Figure 1. TOP I mutation correlated to CPT-11 resistance. NCBI reference sequence: NM_003286.1. (A) Protein sequence; Y, active catalytic tyrosine site; shaded location with underline: mutation site related to TOP I resistance; 1, region 1; 2-1 and 2-1, region 2; 3, region 3; 4, region 4 [see Redinbo *et al* (6) for the numbering of each region].

5 single nucleotide polymorphisms (SNPs; 4 introns, and 1 exon: *UGT1A1**6) within the *UGT1A1* gene and serum total bilirubin levels was noted in Japanese subjects (15).

Although the TOP-I mutation-related CPT-11 resistance has been elucidated, the prevalence of TOP-I germline mutation has yet to be investigated. The prevalence of TOP-I germline mutation requires investigation to elucidate the group of TOP-I-resistant patients prior to cancer chemotherapy. On the other hand, polymorphisms of *UGT1A1* have been revealed to be associated with CPT-11 toxicity in clinical situations. Additionally, less frequent (~50%) *UGT1A1**28 and frequent *UGT1A1**6 have been reported in individuals of Asian descent compared to Caucasians and individuals of African descent (16-21). The prevalence of *UGT1A1* polymorphisms in Asian individuals requires further confirmation studies to avoid toxicity-related death in cancer patients.

The primary aim of this study was to investigate the prevalence of mutation in the TOP-I exons associated with CPT-11 resistance, including untreated cancer tissue. A secondary aim was to confirm the frequency of polymorphisms in the *UGT1A1* genes related to CPT-11 toxicity in a Japanese population.

Materials and methods

Materials. The study protocol was approved by the Ethics Committee of Kitasato University School of Medicine, Japan (B03-07 for healthy volunteers, B03-28 and G03-04 for patients with lung cancer). Blood samples of healthy volunteers (n=236 for TOP-I mutations and n=126 for *UGT1A1* polymorphism) as well as lung cancer tissue specimens from untreated patients (n=16) were examined. The lung cancer tissue samples comprising 6 resected tissues and 10 biopsy specimens constituted of 8 cases of small cell carcinoma, 7 of adenocarcinoma and 1 of squamous cell carcinoma. All of the subjects provided written informed consent prior to their being enrolled in this study.

DNA extraction and polymerase chain reaction. DNA was extracted from 200 μ l whole blood using the QIAamp® DNA blood mini kit (Qiagen, Valencia, CA, USA). Paraffin-embedded lung cancer tissues obtained from lung cancer patients were stained with hematoxylin and eosin, and the DNA was extracted from LASER-captured microdissected tumor tissue (AS LMD, Leica, Tokyo, Japan) using the QIAamp® DNA Micro kit (Qiagen).

Polymerase chain reaction (PCR) was performed to amplify the targeted regions of TOP-I (or *UGT1A1*) with a thermal cycler (Takara Thermal Cycler SP, Takara, Shiga, Japan; or PTC-200 DNA Engine, MJ Research, Watertown, MA, USA) using each primer (Table I), HotStarTaq DNA polymerase and the Q-Solution Kit (Qiagen) in accordance with the manufacturer's protocol, with the exception of the case of *UGT1A1**28 (see below). The PCR products were identified by gel electrophoresis. After excising the DNA fragment band from the agarose gel, high final concentrations of DNA were extracted using a MinElute® gel extraction kit (Qiagen).

Genetic analyses of TOP-I. Five TOP-I mutations correlated to CPT-11 resistance were previously reported (2-7), as follows [see Redinbo *et al* (6) for the numbering of each region]: region 1, exon 20 (541-558); region 2-1, exon 12 (501-530); region 2-2, exon 15 (186-194); region 3, exon 20 (577-579); and region 4, exon 15 (99-101) (Fig. 1A and B). The 5 mutation-hotspot regions of TOP-I were amplified using the relevant primer pairs (Table I). Direct sequencing of the PCR products was performed using ABI PRISM 3100 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA, USA). Sequencing reactions were performed in both the forward and reverse directions.

Genetic analyses of UGT1A1. The following variant sequences were investigated: a 2-extra-nucleotide insertion (TA) within the TATA box in the promoter, resulting in the sequence

B

cDNA sequence

```
1      caaatgagaa cttaggctgt tacacaactg ctggggctgt ttctgcgcgc ccgcccggca
61     gtcaggcagc gtcgcgcgcg tggtagcagc ctccagcgtt ttctgagttc cgggacaca
121    gtcaccgcgc cttacotgog cctcctcgag cctccggagt cccctgcggc ccgcacaaag
181    gcccgtgcgc gtcctgctct ccccacgcgc gcctcgccgt ccgcgcgcgt cgtccctcgc
241    ggcgcgacgt agtggggacc acctccacaa cgtattccag atcgaagcgg atttcogatt
301    gaatgattct cataaacaca aagataaaca caaagatcga gaacaccggc acaaaagaca
361    caagaaggag aaggaccggg aaaagtccaa gcatagcac agtgaacata aagattctga
421    aaagaaacac aaagagaagg agaagaccaa acacaaagat ggaagctcag aaaagcataa
481    agacaaacat aaagacagag acaaggaaaa acgaaaagag gaaaaggttc gagcctctgg
541    ggatgcaaaa ataaagaagg agaaggaaaa tggctctctc agtccaccac aaattaaaga
601    tgaacctgaa gatgaggtgt atttggttcc tcttaagag gatataaagc cattaaagag
661    acctcgagat gaggatgatg ctgattataa acctaaagaa attaaaacag aagataccaa
721    gaaggagaag aaaagaaaac tagaagaaga agaggatggt aaattgaaaa aacccaagaa
781    taagataaaa gataaaaaag ttccgtgagc agataacaag aaaaagaagc cgaagaaga
841    agaggaaacg aagtggaaat ggtgggaaga agagcgtctat cctgaagcga tcaagtggaa
901    attcctagaa cataaaggtc cagtatttgc cccaccatag gacccctctc cagagaatgt
961    caagtcttat tatgatggtg aagtcatgaa gctgagccgc aaagcagagg aagttagctac
1021   gttctttgca aaaatgctgc accatgaata tactaccaag gaaatattta ggaataattt
1081   ctttaagacg tggagaaggg aaatgactaa tgaagagaag aatattatca ccaaccataa
1141   caaatgtgat ttaccacaga tgagccagta tttaaaagcc cagacggagc ctgggaaca
1201   gatgagcaag gaagagaacg tgaaaatcaa agaggagaat gaaaattacg tgaagaata
1261   tggattctgt attatggata acccaaaaga gaggattgct aacttcaaga tagagctcc
1321   tggacttttc artggcgcgc gcaaccaccc caagatgggc atgctgaaga gaagatcat
      Region 2-1
1381   gcccgaggat ataactatca actgtagcaa agatgcacag gttccttctc cctcctcagg
1441   acataagctg aaagaagtcg ggcattgata caaggttact tggctgtgtt cctggacaga
1501   gaacatccaa ggttccatta aatacatcat gcttaacccg agttcacgaa tcaagggtga
1561   gaaggactcg cagaatacgc agactgctcg ggcgtgaaa aaatgtgtgg acaagatcgc
1621   gaaccagtat cgagaagact ggaagtccaa agagatgaaa gtcgggcaga gagctgtagc
1681   cctgtacttc atcgacaagc ttgctctgag agcagcgaat gaaaaggagg aaggagaac
1741   agcggacact gtcggcgtct gctcacttcg tgtggagcac atcaatctac acccagagtt
      Region 4
1801   ggatggtcag gaatagtgg tagagtgtga cttcctcggg aagactcca tcagatacta
      Region 2-2
1861   taacaaggtc cctgttgaga aacgagtttt taagaacctc caactattta tggagaacaa
1921   gcagcccgag gatgactttt ttgatagact caatactggt attctgaata agcatctca
1981   ggatctcatg gagggcttga cagccaaagt attcgtaca tacaatgctc ccatcagctc
2041   acagcagcag ctaaaagaac tgacagcccc ggatgagaac atccagcga agatccttct
2101   ttataacgag gccaatcgag ctggttgaat tctttgtaac catcagaggg caccacacaa
2161   aacttttgag aagtcctatg tgaacttgca aactaagatt gatgccaa gaagaaacagct
2221   agcagatgac cggagagacc tgaaaagtgc taaggctgat gccaaagtca tgaagatgc
2281   aaagacgaag aaggtagtag agtcaaaaga gaaggctgtt cagagactgg aggaacagtt
2341   gatgaagctg gaagtcaag ccacagacgc agaggaaaat aaacagattg ccttggaac
2401   ctccaaactc aattatctgg accctaggat cacagtggct tggctgcaaga agtggggtgt
      Region 1
2461   ccaatttgag aagatttaca acaaaaccca gcgggagaag ttgcctcggg ccattgacat
2521   ggctgatgaa gactatgagt tttagccagt ctcaagagcg agagtctgtt gaagaggac
2581   agtctggttt gggaaagatg gataaactga gcctcacttg cctcgtgccc tgggggagag
2641   aggcagcaag tcttaacaaa ccaacatctt tgcgaaaaga taaccctgga gatattataa
2701   gggagagctg agccagctgt cctatggaca acttatttaa aaatatttca gatatacaaa
2761   ttctagctgt atgatttgtt ttgaattttg tttttatttt caagagggca agtggatggg
2821   aatttgcag gttctacca ggcacattca ctgttccact gaaatgtttg gattctctta
2881   gctactgtat gcaaaagtcg attatatttg tgcgttttta cagttagggt tttgcaataa
2941   cttctatatc ttaataaaaa taaattccta aactcccttc cctctctccc atttcaggaa
3001   tttaaaatta agtagaaca aaaacccagc gcaactgtta gactcgtcac tctctattgt
3061   catgggcatc aattttcatt aaactgaaag cagtgtgtgc ttggcagtg ttttggttca
3121   gacacctgtt acagaaaaa gcatgatggg aaaatatttc ctgacttgag tgtcctttt
3181   taaatgtgaa ttttatctt ttttaattat tttaaaatat ttaaaccttt tctctgatct
3241   taaagatcgt gtatgtggg gttggggagg gatgaaggcg gagtgaatct aaggaataag
3301   aaataatcag tgactgaac cattttccca tcatcctttg ttctgagcat tctgtgac
3361   ctttaagata tccatctttt tcttttaaac cctaatcttt cactgaaag attttattgt
3421   ataaaaagtt tcacaggtca ataaacttag aggaaaatga gtatttggtc caaaaaagg
3481   aaaaataatc aagatttag ggcttttatt tttctttttg taattgtgta aaaaatggaa
3541   aaaaacataa aaagcagaat tttaagtga agacattttt tgcataatc attagtttta
3601   gaggcattgt tagtttagtg tgtgtgcaga gtcattttc cactctcttc ctcaagatc
3661   ttctattttt atcatgaatt ccttttaaat caactgtagg ttatttaaaa ataaattcca
3721   tcaacttaat caaaacttaa
```

Figure 1. (B) cDNA sequence; *tat, active catalytic tyrosine site. Shaded location with bold character(s) and underline: mutation site correlated to TOP I resistance.

(TA)₇TAA (-39 to -53, *UGT1A1**28: rs8175347); transition (+295 from the initial site of transcription, G to A) at codon 71 in exon 1 that is associated with a substitution of glycine to arginine (G71R, *UGT1A1**6: rs4148323); and a transversion (+770, C to A) at codon 229 in exon 1 that alters proline to glutamine (P229Q, *UGT1A1**27: rs35350960). The variant longer sequence of *UGT1A1**28 was distinguished from the wild-type

sequence using poly-acrylamide gel electrophoresis (PAGE) with a DNA-sequencer (Long-Read Tower™, Visible Genetics, Suwanee, GA, USA) following amplification of the targeted sequence using PCR with Pfu DNA Polymerase (native) (Fermentas Life Sciences, Crt Burlington, Ontario, Canada). The primers (Table II) were designed to amplify a 216-bp segment of *UGT1A1**28 variant-type sequence as compared

Table I. Primers for topoisomerase I and *UGT1A1*.

	Forward primer 5'-3'	Reverse primer 5'-3'
TOP-I		
exon20	TAGGGTAGTAGAGTCAAAGAAGAA	GCCAGAAGTTTCCCCAGAGG
exon12	GACTTTCCTCTACCTTGACTTA	GACGCCCTCCACCCCCTTTT
exon15	TTCCATTCATGCTCATCTTTTCTT	TGTGCCTGTTGCCTGTCTCA
<i>UGT1A1</i>		
<i>UGT1A1</i> *6	AAGTAGGAGAGGGCGAACC	GTGGGCAGAGACAGGTACT
<i>UGT1A1</i> *27	AGTACCTGTCTCTGCCAC	GTCCCACTCCAATACACAC
<i>UGT1A1</i> *28	TATAGTCACGTGACACAGTC	CCACTGGGATCAACAGTATCT

TOP-I, topoisomerase I; *UGT1A1*, UDP-glucuronyltransferase 1A1; *UGT1A1**6: G→A on exon 1 (protein, G71R); *UGT1A1**27, 770C→A on exon 1 (protein, P229Q); *UGT1A1**28, (TA)₇TAA on the promoter (protein, reduced expression of *UGT1A1*).

Table II. Co-occurrence of *UGT1A1**28, *UGT1A1**6 and *UGT1A1**27 polymorphisms in healthy volunteers (n=126).

Number of cases among the 126 subjects		<i>UGT1A1</i> *28			Total in <i>UGT1A1</i> *6 or *27
		Wild	Hetero	Homo	
<i>UGT1A1</i> *6	Wild	71	18	3	92
	Hetero	26	4	0	30
	Homo	4	0	0	4
<i>UGT1A1</i> *27	Wild	101	21	3	125
	Hetero	0	1	0	1
	Homo	0	0	0	0
Total in <i>UGT1A1</i> *28		101	22	3	126

UGT1A1, UDP-glucuronyltransferase 1A1 gene; wild, wild-type; hetero, heterozygous; homo, homozygous.

with the 214-bp segment of the wild-type sequence. The separation ability was previously verified using guaranteed wild-type and variant-type genome DNA obtained from Daiichi Pure Chemicals (now Sekisui Medical Co. Ltd., Japan). For analysis of *UGT1A1**6 and *UGT1A1**27, direct sequencing of the PCR amplification product obtained using specific primers (Table I) was performed using the ABI PRISM 3100 genetic analyzer.

Results

Mutations of TOP-I related to CPT-11 resistance. Since no TOP-I mutations were observed in any of the 126 subjects, an additional 110 healthy volunteers, as well as untreated lung cancer tissue specimens in patients (n=16), were investigated. The results revealed that no genetic mutations correlated to CPT11-resistance in exons 12, 15, 16 or 20 of TOP-I.

***UGT1A1* genetic polymorphism associated with CPT-11 toxicity.** Homozygous polymorphisms of *UGT1A1* were detected in 126 subjects, and were distributed as follows: *UGT1A1**28 in 3 (2.4%) subjects and *UGT1A1**6 in 4 (3.2%) subjects (Table II), and co-occurrence of heterozygous polymorphisms for both *UGT1A1**6 and *UGT1A1**28 in 4 subjects

(3.2%), and for both *UGT1A1**27 and *UGT1A1**28 in 1 (0.8%) subject (Table III). The frequency of the *UGT1A1**28 variant allele was found to be 19.8%. In general, either homozygous or heterozygous polymorphisms of *UGT1A1**6 were detected in 30 (23.8%) subjects, and either homozygous or heterozygous polymorphisms of *UGT1A1**6, *27 or *28 were detected in 55 (43.7%) subjects.

The Hardy-Weinberg deviation test showed that *UGT1A1**6 (p=0.43), *27 (p=0.96) and *28 (p=0.19) was not significantly deviated from the equilibrium. The linkage analysis revealed that normalized linkage disequilibrium coefficient D' for *UGT1A1**6 and *UGT1A1**28 was 0.05. However, the linkage disequilibrium was not detected (p=0.64).

Discussion

This is the first study to examine the frequency of TOP-I mutation associated with CPT-11 resistance in healthy subjects and in untreated lung cancer tissue specimens. Although *in vitro* reports have demonstrated that CPT-11-resistant cancer cell lines exposed to CPT-11 possessed TOP-I mutations with no obvious or only minor defects in cell function, these changes were undetectable in healthy subjects and in untreated lung cancer tissue as mutation. Therefore, in the initial chemo-

therapy with CPT-11, the resistance-related TOP-I mutation is unlikely to occur.

Tsurutani *et al* (22) examined 16 samples obtained from 8 CPT-11-treated patients with lung cancer, and detected 2 types of TOP-I mutations in exon 21 in 1 tumor specimen. In a human colon cancer cell line (HCT-15) (23), one exonic mutation was detected in a heterozygous state in exon 19. Since the mutations coded on exon 19 and 21 have not been reported in previous *in vitro* CPT-11-resistant cancer cell lines, these regions were not examined in our study. However, the results indicated that the development of some acquired CPT-11 mutations was possible in patients in the course of treatment with CPT-11.

According to the SNP database (<http://www.ncbi.nlm.nih.gov/SNP/>), missense mutations in exons 4 (1 locus), 9 (1 locus), 15 (3 loci), and 21 (1 locus) are currently registered. These SNPs are not correlated to CPT-11 resistance, although a haplotype-tagging SNP in the intervening sequence region has been found to be associated with toxicity (grade 3/4 neutropenia) in patients treated with CPT-11 (17).

Determination of the *UGT1A1* genotypes is clinically significant for the prediction of CPT-11-related severe toxicity (11). Individuals who have at least 1 variant (heterozygous) allele for the *UGT1A1**28 may exhibit reduced elimination of SN-38 and increased probability of development of dose-limiting neutropenia (11).

The frequency of the *UGT1A1**28 variant allele has been reported to be 30-45% in Caucasian, African and Indian populations, which is approximately twice that of the 10-20% reported in East Asian populations (17-22), as confirmed by the frequency of this study (19.8%). On the other hand, *UGT1A1**6 has been detected in 16-40% of Asian individuals, as confirmed by the frequency of 23.8% found in this study; *UGT1A1**6, however, was extremely rare in the Caucasian and African populations (17). The *UGT1A1**27 allele detected only in Asian individuals was reported to be harboured exclusively by a *UGT1A1**28 haplotype, as findings of this study show. Either homozygous or heterozygous polymorphisms of *UGT1A1**6, *27 or *28 were detected in 55 (43.7%) subjects in this study; the risk of *UGT1A1* polymorphism-related toxicity of CPT-11 is considered to be high in East Asian individuals.

The Food and Drug Administration in the United States has approved an amendment of the label for Camptosar (irinotecan hydrochloride), to which a warning to reduce the starting dose of irinotecan for *UGT1A1**28 homozygous patients has been added. In East Asian individuals, both *UGT1A1**6 and *UGT1A1**28 require examination, since there is no linkage disequilibrium between the two polymorphisms (24), as indicated in the present study.

As a clinical relevance, the risk of TOP-I mutation-related resistance to CPT-11 is unlikely and it is not necessary to test for TOP-I mutation prior to chemotherapy with CPT-11. However, the risk of *UGT1A1* polymorphism-related toxicity of CPT-11 is markedly higher in East Asian individuals, and *UGT1A1* polymorphisms, not only of *UGT1A1**28, but also of *UGT1A1**6, should be tested prior to treatment with CPT-11 to avoid severe adverse effects.

In conclusion, the main findings in this study were twofold. First, TOP-I genetic mutations related to CPT-11 resistance

were not detected in any of the subjects or in untreated lung cancer tissues. Second, compared to Caucasians and individuals of African descent, it was observed that *UGT1A1**28 was less frequent and *UGT1A1**6 was more frequent in Japanese subjects. Moreover, linkage disequilibrium was not noted between *UGT1A1**6 and *UGT1A1**28.

Acknowledgements

We thank Yakult Honsha Co., Ltd., and Masumi Tanaka (Department of Respiratory Medicine) for their partial technical support. This study was supported by Grant-in-Aid No. 15590826 and Grant-in-Aid for Development of leading-edge cancer professionals in the South Kanto area, from the Ministry of Education, Culture, Sports, Science and Technology.

References

- Pizzolato JF and Saltz LB: The camptothecins. *Lancet* 361: 2235-2242, 2003.
- Arakawa Y, Suzuki H, Saito S and Yamada H: Novel missense mutation of the DNA topoisomerase I gene in SN-38-resistant DLD-1 cells. *Mol Cancer Ther* 5: 502-508, 2006.
- Kubota N, Kanzawa F, Nishio K, Takeda Y, Ohmori T, Fujiwara Y, Terashima Y and Saijo N: Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. *Biochem Biophys Res Commun* 188: 571-577, 1992.
- Chang JY, Liu JF, Juang SH, Liu TW and Chen LT: Novel mutation of topoisomerase I in rendering cells resistant to camptothecin. *Cancer Res* 62: 3716-3721, 2002.
- Chrencik JE, Staker BL, Burgin AB, Pourquier P, Pommier Y, Stewart L and Redinbo MR: Mechanisms of camptothecin resistance by human topoisomerase I mutations. *J Mol Biol* 339: 773-784, 2004.
- Redinbo MR, Stewart L, Kuhn P, Champoux JJ and Hol WGJ: Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. *Science* 279: 1504-1513, 1998.
- Laco GS, Collins JR, Luke BT, Kroth H, Sayer JM, Jerina DM and Pommier Y: Human topoisomerase I inhibition: docking camptothecin and derivatives into a structure-based active site model. *Biochemistry* 41: 1428-1435, 2002.
- Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G and Sparreboom A: Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin Cancer Res* 7: 2182-2194, 2001.
- Humerickhouse R, Lohrbach K, Li L, Bosron WF and Dolan ME: Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. *Cancer Res* 60: 1189-1192, 2000.
- Kawato Y, Aonuma M, Hirota Y, Kuga H and Sato K: Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. *Cancer Res* 51: 4187-4191, 1991.
- Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K and Hasegawa Y: Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Res* 60: 6921-6926, 2000.
- Ando Y, Fujita K, Sasaki Y and Hasegawa Y: *UGT1A1**6 and *UGT1A1**27 for individualized irinotecan chemotherapy. *Curr Opin Mol Ther* 9: 258-262, 2007.
- Araki K, Fujita K, Ando Y, Nagashima F, Yamamoto W, Endo H, Miya T, Kodama K, Narabayashi M and Sasaki Y: Pharmacogenetic impact of polymorphisms in the coding region of the *UGT1A1* gene on SN-38 glucuronidation in Japanese patients with cancer. *Cancer Sci* 97: 1255-1259, 2006.
- Strassburg CP: Pharmacogenetics of Gilbert's syndrome. *Pharmacogenomics* 9: 703-715, 2008.
- Saito A, Kawamoto M and Kamatani N: Association study between single-nucleotide polymorphisms in 199 drug-related genes and commonly measured quantitative traits of 752 healthy Japanese subjects. *J Hum Gen* 54: 317-323, 2009.

16. Kaniwa N, Kurose K, Jinno H, Tanaka-Kagawa T, Saito Y, Saeki M, Sawada K, Tohkin M and Hasegawa R: Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide polymorphism 686C> T (P229L) found in an African-American. *Drug Metab Dispos* 33: 458-465, 2005.
17. Hoskins JM, Marcuello E, Altes A, Marsh S, Maxwell T, Van Booven DJ, Paré L, Culverhouse R, McLeod HL and Baiqet M: Irinotecan pharmacogenetics: influence of pharmacodynamic genes. *Clin Cancer Res* 14: 1788-1796, 2008.
18. Chowbay B, Zhou S and Lee EJ: An interethnic comparison of polymorphisms of the genes encoding drug-metabolizing enzymes and drug transporters: experience in Singapore. *Drug Metab Rev* 37: 327-378, 2005.
19. Mercke Odeberg J, Andrade J, Holmberg K, Hoglund P, Malmqvist U and Odeberg J: UGT1A polymorphisms in a Swedish cohort and a human diversity panel, and the relation to bilirubin plasma levels in males and females. *Eur J Clin Pharmacol* 62: 829-837, 2006.
20. Liu JY, Qu K, Sferruzza AD and Bender RA: Distribution of the UGT1A1*28 polymorphism in Caucasian and Asian populations in the US: a genomic analysis of 138 healthy individuals. *Anticancer Drugs* 18: 693-696, 2007.
21. Innocenti F, Grimsley C, Das S, Ramirez J, Cheng C, Kuttub-Boulos H, Ratain MJ and Di Rienzo A: Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. *Pharmacogenetics* 12: 725-733, 2002.
22. Tsurutani J, Nitta T, Hirashima T, Komiya T, Uejima H, Tada H, Syunichi N, Tohda A, Fukuoka M and Nakagawa K: Point mutations in the topoisomerase I gene in patients with non-small cell lung cancer treated with irinotecan. *Lung Cancer* 35: 299-304, 2002.
23. Moisan F, Longy M, Robert J and Le Morva V: Identification of gene polymorphisms of human DNA topoisomerase I in the National Cancer Institute panel of human tumour cell lines. *Br J Cancer* 95: 906-913, 2006.
24. Saito Y, Maekawa K, Ozawa S and Sawada J: Genetic polymorphisms and haplotypes of major drug metabolizing enzymes in east Asians and their comparison with other ethnic populations. *Curr Pharmacogenomics* 5: 49-78, 2007.