表1　集積全症例（n=118）

	FOLFOX (n=77)	FOLFIRI (n=41)	計
初回治療	26 (33.8)	10 (24.4)	36 (30.5)
二次治療	17 (22.1)	5 (12.2)	22 (18.6)
三次治療～	34 (44.2)	26 (63.4)	60 (50.8)

(%)

サリプラチン（商品名：エルプラット®，以下L-OHP）が前述のℓ-LV/5-FU持続レジメンとの併用で承認され，FOLFOX療法[5]が施行可能になった。

今回FOLFOX療法，およびFOLFIRI療法の施行が可能になって約1年が経過したことから，北信癌化学療法談話会幹事施設および診療科において実施された各療法の有効性，有害事象について後ろ向き解析を行った。

I　対象および方法

1．対象

2005年3月から2006年3月までに当会幹事施設において進行大腸癌に対しFOLFOX療法，またはFOLFIRI療法が施行された症例は計118例であった。このうち，初回治療，または二次治療として施行された58例を今回の有効性，有害事象についての評価対象とした（表1）。なお，初回治療としてFOLFOX療法を行った症例のうち4例は評価に至らず，評価対象は54例であった。

2．投与方法

本邦で施行可能なFOLFOX療法には，FOLFOX4 regimen[6]とmFOLFOX6 regimenの2つの投与方法がある。FOLFOX4 regimenは2週間に1度，2日間かけて行う方法であり，day 1にℓ-LV 100mg/m²とL-OHP 85mg/m²を2時間で静注後，5-FU 400mg/m²をbolus，さらに5-FU 600mg/m²を22時間で持続点滴し，day 2にはL-OHPを除きday 1と同じ投与を繰返す方法である。mFOLFOX6 regimenはこれを簡略化したもので，ℓ-LV 200mg m²とL-OHP 85mg/m²を2時間で静注後，5-FU 400mg·m²をbolus，さらに5-FU 2400～3000mg/m²を46時間で持続点滴する方法である（図1）。今回FOLFOX4 regimenが3例に，mFOLFOX6 regimenが40例に施行されていた。なお，L-OHPの末梢神経障害を予防するために，原則としてL-OHP投与の前後にCa，Mg製剤が投与[7][8]されていた（図2）。

代表的なFOLFIRI regimenの投与方法は，mFOLFOX6 regimenのL-OHPの部分をCPT-11 150～180mg/m²の90分点滴に代えて，ℓ-LVと同時に投与を開始するものである（図1）。

なお，FOLFOX療法，FOLFIRI療法の投与は原則として中心静脈ポートの埋込みで行われている。

3．評価・確認項目

以下について評価を行った。

・症例数，患者背景
・二次治療群における前治療
・投与法，初回治療，二次治療毎の有効性
・有害事象

なお，治療効果はRECISTガイドライン[9]の判定基準に準拠した。また，有害事象の評価にはCTCAE v3.0日本語訳JCOG/JSCO版[10]を用いた。ただし，末梢神経障害についてはDEBNTC（Neurotoxicity criteria of DEBIOPHARM)[11]で判定した（表2）。

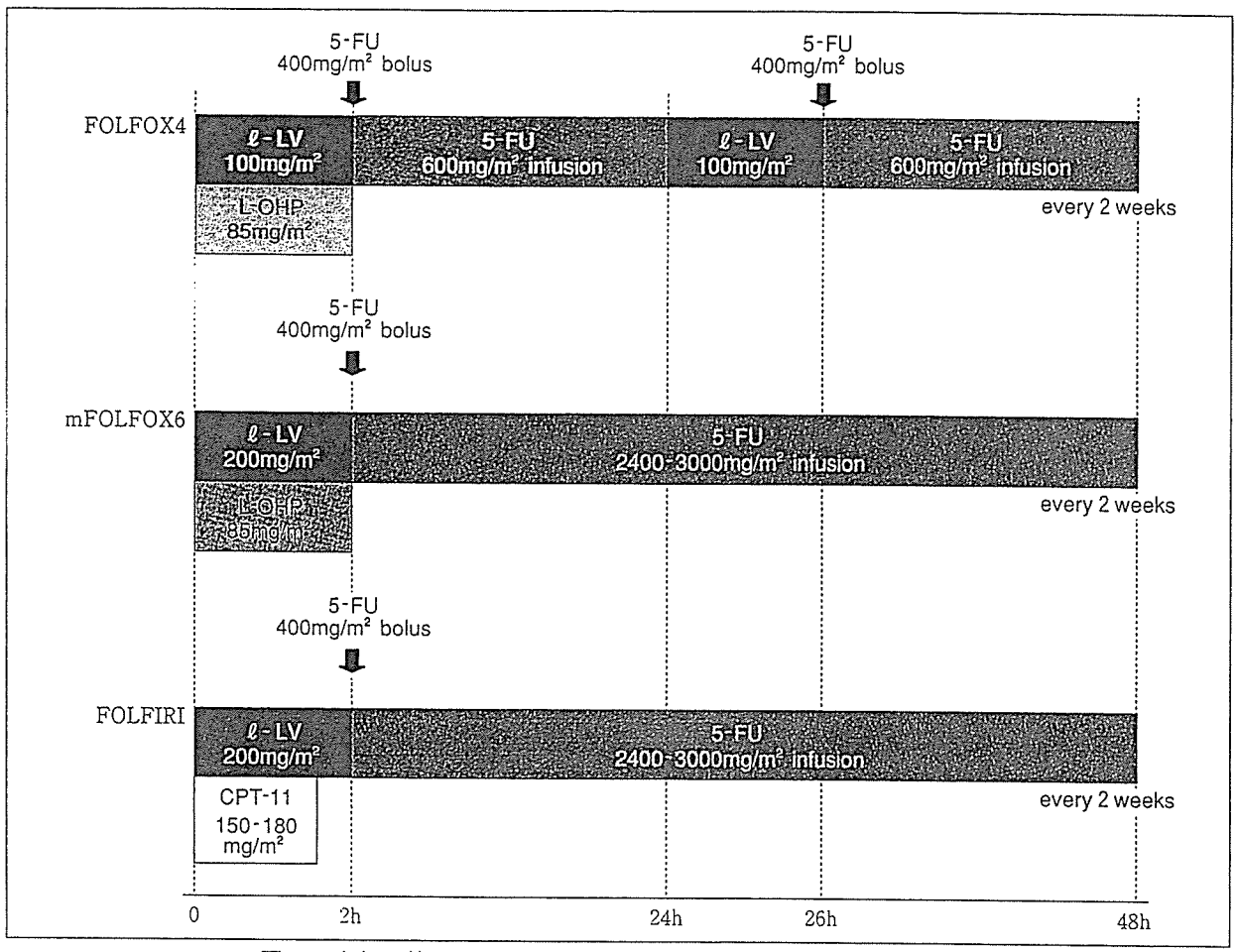

図1　本邦で施行可能なFOLFOX, FOLFIRI の投与スケジュール

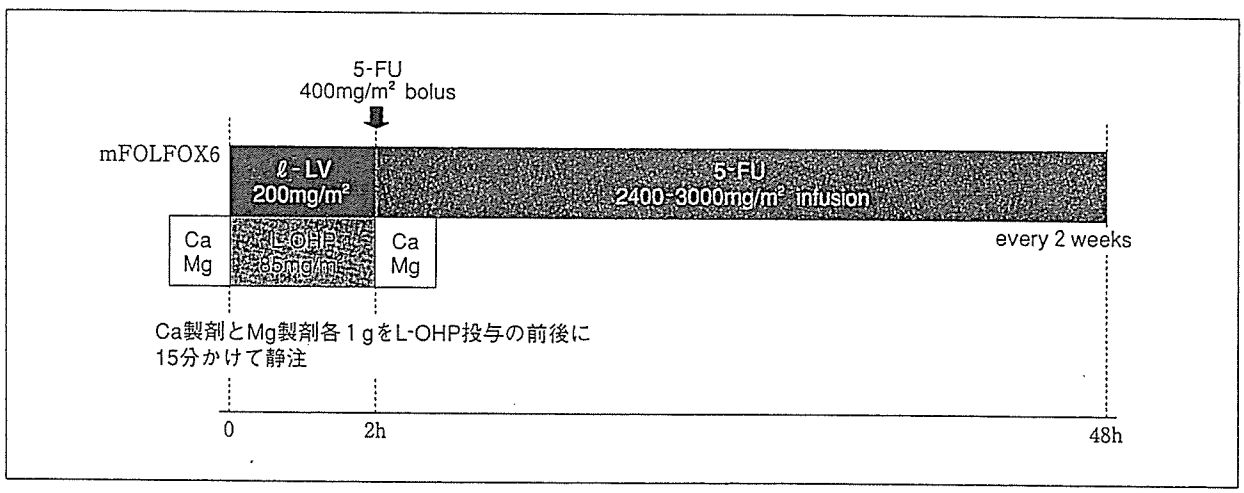

図2　末梢神経障害対策（例）

表2　知覚異常/感覚異常の評価

CTCAE v3.0
G1：症状がない；深部腱反射消失または知覚異常（疼きを含む）があるが機能障害はない
G2：知覚変化または知覚異常（疼きを含む）による機能障害はあるが，日常生活に支障がない
G3：日常生活に支障がある知覚変化または知覚異常
G4：活動不能，動作不能
DEB-NTC
G0：異常なし
G1：末梢神経症状の発現。ただし7日未満で消失
G2：7日以上持続する末梢神経症状。ただし機能障害はない
G3：機能障害の発現

表3　解析症例の内訳

		FOLFOX	FOLFIRI
No. of Pts		39	15
age, years	median (range)	61 (26-83)	67 (49-75)
sex	F/M	19/20	4/11
PS	0/1/2/3	20/16/2/1	10/4/0/1
location	C/A/T/D/S/R	3/2/4/0/10/20	1/3/2/1/5/3
cycle	median (range)	6 (2-14)	7 (1-21)

Ⅱ　結　　果

1．症例数，患者背景

集積された全症例はFOLFOX77例，FOLFIRI41例の合計118例（表1）であった。その内訳は初回治療36例（30.5%）二次治療22例（18.6%）三次治療以降60例（50.8%）であり，FOLFOX症例の44.2%，FOLFIRI症例の63.4%は三次治療以降であった。

初回治療または二次治療として施行した症例は58例であったが，FOLFOX症例のうち初回治療の4例が評価に至らず，評価可能症例はFOLFOX症例39例，FOLFIRI症例15例の計54例であった（表3）。FOLFOX症例のPerformance Status（PS）は2例を除いて0～2であった。PS3の2例は本人および家族の強い希望があり施行した。

2．二次治療群における前治療

二次治療としてFOLFOX療法またはFOLFIRI療法が施行された22例について，その前治療を確認した（表4）。FOLFOX症例の前治療はFOLFIRIが1例，IFL[12)13)]が6例，RPMI[14)]が4例，UFT/LV[15)]が5例，その他が1例であった。FOLFIRI症例の前治療ではRPMIが3例，UFT/LVが1例，その他が1例であった。

3．有効性

初回治療としてFOLFOX療法を施行した症例の奏効率は50.0%（11/22）であり，SD症例まで含めると86.4%（19/22）の腫瘍制御率であった。二次治療群での奏効率は23.5%（4/17）と初回治療群の半分以下であったが，SD症例

表4　二次治療群における前治療の内訳

	FOLFOX (n=17)	FOLFIRI (n=5)
FOLFOX		0
FOLFIRI	1	
IFL	6	0
RPMI	4	3
UFT/LV	5	1
other	1	1

表5　FOLFOX，FOLFIRI療法の成績

	FOLFOX (n=39)		FOLFIRI (n=15)	
	奏効率	腫瘍制御率	奏効率	腫瘍制御率
初回治療	50.0% (11/22) CR 2 /PR 9	86.4% (19/22) SD 8 (PD 3)	40.0% (4/10) CR 1 /PR 3	50.0% (5/10) SD 1 (PD 5)
二次治療	23.5% (4/17) CR 0 /PR 4	76.5% (13/17) SD 9 (PD 4)	20.0% (1/5) CR 0 /PR 1	60.0% (3/5) SD 2 (PD 2)

が9例と多く，腫瘍制御率は76.5% (13/17) であった。一方，初回治療としてFOLFIRI療法を施行した症例での奏効率は40.0% (4/10)，腫瘍制御率は50.0% (5/10) であった（表5）。

4．有害事象

FOLFOX療法におけるGrade 3 以上の有害事象発現率は，初回治療群で19.2% (5/26) であったのに対し，二次治療群では70.6% (12/17) であった。L-OHPまたは5-FUを減量されている症例は初回治療群で30.8% (8/26)，二次治療群では52.9% (9/17) であった。

FOLFOX療法について最も多かった有害事象は，白血球減少14例であった。他に血小板減少1例，意識障害1例，食欲不振1例が認められた。また，Grade 3 以上の末梢神経障害は認められなかった。

一方，FOLFIRI療法でのGrade 3 以上の有害事象発現率は初回治療群で60.0% (6/10)，二次治療群では40.0% (2/5) であった。CPT-11または5-FUが減量されている症例は初回治療群，二次治療群ともに各1例であった。最も多かった有害事象は白血球減少6例であり，他にDIC 1 例と脳梗塞1例が認められた。なおFOLFOX療法，FOLFIRI療法ともに治療関連死はなかった（表6）。

5．まとめ

当会の幹事施設および診療科において約1年の間にFOLFOX療法，またはFOLFIRI療法が施行された症例について検討した。全体の約半数が三次治療以降の症例であったが，今回は初回治療と二次治療の有効性と有害事象を確認した。初回治療と二次治療での治療法の選択は，FOLFOX療法がFOLFIRI療法の3倍近く行われていた。今回の集積は2005年3月からであったが，二次治療症例での前治療は，ほとんどの症例は大腸癌治療ガイドライン[16]で推奨されている治療法の範囲（RPMI 7例，IFL 6例，UFT/LV 6例，FOLFIRI 1例，

表6　Grade 3 以上の有害事象

	FOLFOX		FOLFIRI	
	初回治療群 (n=26)	二次治療群 (n=17)	初回治療群 (n=10)	二次治療群 (n=5)
有害事象	5 (19.2)	12 (70.6)	6 (60.0)	2 (40.0)
減量投与	8 (30.8)	9 (52.9)	1 (10.0)	1 (20.0)
白血球減少	4	10	4	2
血小板減少	1			
意識障害		1		
食欲不振		1		
DIC			1	
脳梗塞			1	

(%)

表7　海外臨床試験におけるFOLFOX，FOLFIRI療法の成績

regimen	phase	RR (%)	PFS (m)	OS (m)	reference
FOLFOX4	Ⅲ	50	8.2	16.2	6)
FOLFOX4	Ⅲ (N9741)	45	8.7	19.5	17)
FOLFOX4	Ⅲ (OPTIMOX1)	59	9.0	19.3	18)
FOLFOX6	Ⅲ (C97)	54	8.0	20.6	5)
FOLFOX7	Ⅲ (OPTIMOX1)	59	8.7	21.2	18)
FOLFIRI	Ⅱ (3rd line)	6	18w	43w	19)
Douillard	Ⅲ	49	6.7	17.4	20)
FOLFIRI	Ⅲ (C97)	56	8.5	21.5	5)

その他2例）であった。FOLFOX療法，FOLFIRI療法ともに初回治療での施行により有効性，有害事象ともに二次治療より良好な結果が得られた。

考　　察

北信癌化学療法談話会は長野県の北部，および東部地区の医師，薬剤師，看護師による，主に消化器癌の化学療法の研鑽を目的とした会であり，年2回の研究講演会等を行っている。今回はFOLFOX，FOLFIRI療法を開始して約1年が経過することから，現状の分析，確認のために症例集積を行った。今回の集積の中では，初回治療でのFOLFOX療法の奏効率は50.0%であり，海外の臨床試験（表7）と同等の結果が実地臨床で得られた。

2004年のTournigandら[5]の報告によると，初回治療でのFOLFOX療法の奏効率は54%，FOLFIRI療法は56%であった。その中でTournigandらはdown stagingによって治癒切除に至った症例を解析しており，FOLFOX療法により治癒切除に至った症例は22%，その生存期間中央値は47カ月でさらに延長中とのことであった。今後，bevacizumab等の分子標的治

療薬の併用によりさらに成績は向上すると考えられ，化学療法の意義が生存期間の延長のみではなくなることも期待される。

その一方で，現在は施行可能で，かつ有効な化学療法をより円滑に実施することが重要と思われる。今回の集積では，FOLFOX症例においてGrade 3 以上の末梢神経障害の発現はなかったが，これは投与回数中央値が 6 回（2～14回）と少なかったこと，Ca，Mg製剤の併用[5]が普及[21]していたことが影響していると考えられた。今後さらに長期間の経過観察を行い，Ca，Mg製剤の末梢神経障害抑制効果について検討を加えたい。他にFOLFOX療法を円滑に施行するためにすでに当会で実施している工夫として，5-FUのbolus投与を10分～15分程度かけて緩序に投与[21]することが挙げられる。また，FOLFOX療法の投与法は多くの場合mFOLFOX6 regimenが選択されていたが，その選択理由はインフューザーポンプを使用することにより 2 日目に来院する必要がなく，外来投与に移行しやすいことがある。さらに，嘔吐や白血球減少の一因とされる5-FUのbolus投与を 1 回に減らすことができ，5-FUの総投与量を増やせるという利点がある。

今回の集積はFOLFOX，FOLFIRI療法を開始してから約 1 年であり生存中の症例がほとんどのため，生存期間に関する解析はできなかった。今後の症例集積では有効性，有害事象の他に生存期間，治癒切除率について検討していく予定である。

参　考　文　献

1）de Gramont, A., Bosset, J., Milan, C. et al.：Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer：A French intergroup study. J. Clin. Oncol. 15 (2) 808, 1997.

2）Tournigand, C., de Gramont, A., Louvet, C. et al.：A simplified bi-monthly regimen with leucovorin (LV) and 5-fluorouracil (5FU) for metastatic colorectal cancer (MCRC), (Meeting abstract) Proc. ASCO 1998, #1052

3）Kohne, C.H., Wils, J., Lorenz, M. et al.：Randomized phase Ⅲ study of high-dose fluorouracil given as a weekly 24-hour infusion with or without leucovorin versus bolus fluorouracil plus leucovorin in advanced colorectal cancer：European Organization of Research and Treatment of Cancer Gastrointestinal Group Study 40952, J. Clin. Oncol. 21 (20) 3721～3728, 2003.

4）抗がん剤併用療法に関する検討会：抗がん剤報告書，フルオロウラシル及びアイソボリン（大腸がん）

5）Tournigand, C., Andre, T., Achille, E. et al.：FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer：A randomized GERCOR study, J. Clin. Oncol. 22, 229～237, 2004.

6）de Gramont, A., Figer, A., Seymour, M. et al.：Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J. Clin. Oncol. 18, 2938～2947, 2000.

7）Gamelin, L., Celle, M. B. et al.：Prevention of oxaliplatin-related neurotoxicity by calcium and magnesium infusions：A retrospective study of 161 patients receiving oxaliplatin combined with 5-fluorouracil and leucovorin for advanced colorectal cancer, Clinical Cancer Research 10, 4055～4061, 2004.

8）Grothey, A.：Clinical management of oxaliplatin-associated neurotoxicity, Clinical Colorectal Cancer 5 (Suppl. 1) S38～46, 2005.

9）Therasse, P., Arbuck, S.G., Eisenhauer, E.A. et al.：New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment for Cancer,

National Cancer Institute of the United States, National Cancer Institute of Canada, J. Natl. Cancer Inst. 92, 205～216, 2000.

10) JCOGデータセンター：福田治彦，佐藤暁洋ほか：CTCAE v3.0 日本語訳 JCOG/JSCO版，大野竜三ほか，日本癌治療学会-癌治療効果判定基準作成委員会，2004.

11) Becouarn, Y., Ychou, M., Ducreux, M. et al.：Phase II trial of oxaliplatin as first-line chemotherapy in metastatic colorectal cancer patients, J. Clin. Oncol. 16 (8) 2739～2744, 1998.

12) Saltz, L.B., Cox, J.V., Blanke, C. et al.：Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group, N. Engl. J. Med. 343, 905～914, 2000.

13) 三嶋秀行，西庄 勇ほか：進行再発大腸癌に対する標準化学療法，消化器科 36 (4) 397～402, 2003.

14) Petrelli, N., Douglass, H.O., Herrera, L. et al.：The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma：a prospective randomized phase Ⅲ trial, J. Clin. Oncol. 7, 1419～1426, 1989.

15) Douillard, J. Y., Hoff, P. M., Skillings, J. R. et al.：Multicenter phase Ⅲ study of uracil/tegafur and oral leucovorin versus fluorouracil and leucovorin in patients with previously untreated metastatic colorectal cancer, J. Clin. Oncol. 20 (17) 3605～3616, 2002.

16) 大腸癌治療ガイドライン 医師用 2005年版，大腸癌研究会編 http://www.jsccr.jp/guideline/index.html

17) Goldberg, R.P., Sargent, D.J., Morton, R.F. et al.：N9741：A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer, J. Clin. Oncol. 22, 23～30, 2004.

18) Tournigand, C., Cervantes, A., Figer. A. et al.：OPTIMOX1：A randmized study of FOLFOX4 or FOLFOX7 with oxaliplatin in a Stop-and-Go Fashion in advanced colorectal cancer －A GERCOR Study, J. Clin. Oncol. 24, 394～400, 2006.

19) Andre, T., Louvet, C., Maindraut-Goebel, F. et al.：CPT-11 (irinotecan) addition to bimonthly, high-dose leucovorin and bolus and continuous-infusion 5-fluorouracil (FOLFIRI) for pretreated metastatic colorectal cancer. GERCOR. Eur. J. Cancer 35, 1343～1347, 1999.

20) Douillard, J. Y., Cunningham, D., Roth, A.D. et al.：Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer：a multicentre randomised trial, Lancet 355, 1041～1047, 2000.

21) 宗像康博，宮田佳典ほか：市中病院における大腸癌化学療法の現状と問題点，Prog. Med. 26, 1057～1064, 2006.

MDR1 T-129C Polymorphism can be Predictive of Differentiation, and Thereby Prognosis of Colorectal Adenocarcinomas in Japanese

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The expression level of MDR1 mRNA was evaluated in colorectal adenocarcinomas and adjacent noncancerous colorectal tissues obtained from 21 Japanese patients. It was lower in the former than in the latter ($p=0.012$), suggesting its down-regulation as a consequence of malignant transformation of colorectal tissues, possibly with the suppression of differentiation. Relatively lower expression was suggested in moderately-differentiated colorectal adenocarcinomas than well-differentiated ones, but there was no statistical difference ($p=0.111$). MDR1 mRNA up-regulation was found in a colorectal adenocarcinoma cell line, HCT-15, after treatment with two typical differentiating agents, sodium butyrate and all-*trans* retinoic acid, suggesting its involvement in the cellular events, resulting in differentiation without malignant transformation. *MDR1* T-129C, but not G2677A,T and C3435T, was associated with the lower expression of MDR1 mRNA both in colorectal adenocarcinomas ($p=0.040$) and adjacent noncancerous colorectal tissues ($p=0.023$), possibly being an useful invasive marker predicting poorly-differentiated colorectal adenocarcinomas and thereby the poor prognosis of the patients, especially when no extra biopsy samples will be obtained. Further investigations with relatively large number of patients should be undertaken to confirm these preliminary results.

Key words colorectal adenocarcinoma; MDR1; expression; differentiation; genetic polymorphism

Multidrug resistant transporter MDR1/P-glycoprotein, the gene product of *MDR1*, is a glycosylated membrane protein of 170 kDa, belonging to the ATP-binding cassette superfamily of membrane transporters.[1—5] MDR1 was originally isolated from resistant tumor cells as part of the mechanism of multidrug resistance,[6—8] and a number of clinical investigations have suggested that the intrinsic or acquired overexpression of MDR1 in tumors resulted in a poor clinical outcome of cancer chemotherapy.[9] Moreover, over the last decade, it has been elucidated that human MDR1 is also expressed throughout the body to confer intrinsic resistance to the tissues by exporting unnecessary or toxic exogeneous substances or metabolites.[10] Various types of structurally unrelated drugs are substrates for MDR1, and MDR1 and other transporters are recognized as an important class of proteins for regulating pharmacokinetics and pharmacodynamics.[1—5] Furthermore, recent investigations have challenged the notion that MDR1 has evolved merely to facilitate the efflux of xenobiotics and have raised the possibility that MDR1 plays a fundamental role in regulating apoptosis.[11,12] Given the down-regulation of MDR1 expression during the differentiation of pluripotent stem cells along the myeloid lineage,[13] its potential implication in cell systems resulting in cell death or differentiation has been discussed for the last decade.

Numerous clinicopathological factors have been reported to have prognostic significance for colorectal cancer, including tumor invasion, nodal metastasis, differentiation and lymphocytic infiltration.[14] The importance of differentiation was already suggested in the 1920s, and the tumors have been graded into well-, moderately- and poorly-differentiated types. Most of colorectal cancers are assessed as well- or moderately-differentiated adenocarcinoma in the Japanese, being more frequently found than Caucasians, and the 5-year survival rate was reported to be 60—72%.[15,16] In contrast, it was only 32—46% for poorly-differentiated adenocarcinoma, although we rarely encountered this.[15,16] Thus, it is important to evaluate the differentiation grade accurately to decide on the patient management strategy; however, its usefulness is sometimes thought to be limited due to difficulties in the assessment and thereby reproducibility, encouraging us to search for alternative molecular markers,[17] or to establish a method of subclassification.[16]

In this study, the expression levels of MDR1 mRNA were assessed in colorectal adenocarcinomas and adjacent noncancerous colorectal tissues obtained from 21 Japanese patients. Here, a relatively low expression level was suggested in moderately-differentiated adenocarcinomas than well-differentiated ones, but there were no patients with poorly-differentiated adenocarcinomas, and the study was not sufficiently powerful to reach statistical significance, and their relationship was attempted to be replicated in vitro using a colorectal adenocarcinoma cell line, HCT-15. The effects of genetic polymorphisms of the *MDR1* gene were also assessed, including T-129C, G2677A,T and C3435T, since they are re-

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ported to affect the expression of MDR1 and its mRNA,[1—5] and therefore possibly become novel and invasive markers predictive of prognosis.

MATERIALS AND METHODS

Human Colorectal Adenocarcinomas and Adjacent Noncancerous Colorectal Tissues Colorectal adenocarcinomas were obtained as surgical samples from 21 Japanese patients with primary colorectal adenocarcinoma diagnosed at Kobe University Hospital (10 men and 11 women). The average age was age 65.9±10.8 years (±S.D.; range, 29—79 years). Adjacent noncancerous colorectal tissues were simultaneously taken, and immediately after resection, these tissue samples were quickly stripped of connective tissue, snap-frozen and stored at −80 °C until processing. Informed consent was obtained from all subjects prior to their participation in the study. The protocol was approved by the Institutional Review Broad of Kobe University Hospital, Kobe University, Japan.

***MDR1* Genotyping** The colorectal adenocarcinomas and adjacent noncancerous colorectal tissues were cut up into small pieces in 1.5-ml microcentrifuge tubes, and then genomic DNA was extracted using a DNeasy Tissue Kit® (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. In this study, *MDR1* genotypes of C-145G (noncoding), T-129C (noncoding), G2677A,T (Ala893Thr, Ser) and C3435T (silent) were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described previously,[18—21] and were confirmed by direct sequencing using an automatic ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.). The sequences of PCR primes for C-145G were 5′-TCA GCA TTC AGT CAA TCC GG-3′ (sense) and 5′-AGT AGC TCC CAG CTT TGC-3′ (anti-sense), and those for T-129C, G2677A,T and C3435T were described previously.[18—21] These primers were synthesized by Hokkaido System Science, Co., Ltd. (Sapporo, Japan).

MDR1 mRNA Levels in Colorectal Adenocarcinomas and Adjacent Noncancerous Colorectal Tissues Assessed by Real-Time Quantitative Reverse Transcription (RT)-PCR Total RNA was extracted from colorectal adenocarcinomas and adjacent noncancerous colorectal tissues using a RNeasy Mini kit (QIAGEN) and a RNase-Free DNase Set (QIAGEN) according to the manufacturers' protocols. The expression levels of MDR1 mRNA in were assessed by real-time quantitative RT-PCR analysis as described previously.[20—24] The sequences of primers for MDR1 were 5′-GCT CAG ACA GGA TGT GAG TTG GT-3′ (forward) and 5′-CCT GGA ACC TAT AGC CCC TTT AAC-3′ (reverse), and these primers were synthesized by Hokkaido System Science, Co., Ltd. The sequence of the TaqMan probe was 5′-AAA AAC ACC ACT GGA GCA TTG ACT ACC AGG-3′, and the probe was synthesized by Operon Biotechnologies, Inc. (Tokyo, Japan). The primers and probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an endogenous RNA control to normalize for differences in the amount of total RNA, were purchased from Applied Biosystems (TaqMan GAPDH Control Reagent Kit). In each run of the assay, the mRNA levels of GAPDH and MDR1 were analyzed in 4- or 5-fold serially diluted samples from an authentic human colon carcinoma cell line, Caco-2, and the assay was validated using a synthetic DNA template. The mRNA levels of MDR1 were expressed as a concentration relative to GAPDH mRNA.

Effect of Differentiating Agents on MDR1 mRNA Expression in a Colorectal Adenocarcinoma Cell Line, HCT-15 HCT-15 (passage 43) was purchased from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). HCT-15 cells were maintained in RPMI1640 culture medium (Invitrogen Corp., Carlsbad, CA, U.S.A.) supplemented with heat-inactivated 10% fetal bovine serum (FBS; CELLect® GOLD, MP Biomedicals, Irvine, CA, U.S.A.). The cells seeded at a density of 3.0×10^6 cells in 40 ml of culture medium in 175 cm^2 culture flasks (Nunclon™, Nalge Nunc International, NY, U.S.A.) were grown in an atmosphere of 95% air and 5% CO_2 at 37 °C, and subcultured every 3—4 d using a mixture of 0.02% EDTA and 0.05% trypsin (Invitrogen Corp.).

HCT-15 cells seeded at a density of 4×10^5 cells in 2 ml of culture medium in a 6-well plate (Nunclon™, Nalge Nunc International) were grown in an atmosphere of 95% air and 5% CO_2 at 37 °C. One day after, the culture medium was replaced, and an aqueous solution of sodium butyrate (NaB) or a dimethylsulfoxide (DMSO) solution of all-*trans* retinoic acid (ATRA), typical differentiating agents, was added to give the final concentrations of 0.1 or 1 mM for NaB and 1 or 10 μM for ATRA. The volume concentration of purified water or DMSO was less than 0.1%. After incubation for another 1 d at 37 °C, the cells were washed twice with ice-cold phosphate buffered saline, and then the cell pellets were prepared and stored at −80 °C until processing. The expression levels of MDR1 mRNA were evaluated as described above.

Statistical Analysis Values are given as the mean±standard deviation (S.D.). The statistical significance of differences between the mean values of MDR1 mRNA levels in colorectal adenocarcinomas and adjacent noncancerous colorectal tissues were calculated using the Wilcoxon signed-rank test. The effects of differentiation or *MDR1* genotypes on the expression levels of MDR1 mRNA were analyzed by multiple comparisons with ANOVA followed by the Scheffé test, or by an unpaired Student's *t*-test. p values less than 0.05 were considered significant.

RESULTS

Figure 1 shows the relative concentrations of MDR1 mRNA in colorectal adenocarcinomas and adjacent noncancerous colorectal tissues obtained from 21 Japanese patients. The expression levels were lower in colorectal adenocarcinomas (3.48±5.97, range 0.09—23.67) than adjacent noncancerous colorectal tissues (12.98±18.85, range 0.11—67.71, $p=0.012$ (Wilcoxon signed-rank test)) (Table 1). The ratio of expression in colorectal adenocarcinomas to adjacent noncancerous colorectal tissues was varied from 0.00 to 6.11 with an average of 0.87±1.50. Histological examination of colorectal adenocarcinomas showed that they consisted of well-differentiated ($n=5$), moderately-differentiated ones ($n=12$) and others ($n=4$). There were no patients with poorly-differentiated adenocarcinomas. As shown in Table 1, the relatively low expression level of MDR1 mRNA was suggested in moderately-differentiated adenocarcinomas (1.13±1.10) than well-differentiated (6.70±6.11, $p=0.111$).

To elucidate the association of MDR1 expression with differentiation, a colorectal adenocarcinoma cell line, HCT-15 was treated with two typical differentiating agents, NaB or ATRA, and as shown in Fig. 2, it was confirmed that MDR1 mRNA was up-regulated after their treatment.

Table 2 summarizes the effects of *MDR1* T-129C, G2677A,T and C3435T on the MDR1 mRNA expression in the colorectal adenocarcinomas and adjacent noncancerous colorectal tissues. The genetic polymorphism of C-145G was not detected in this study. The expression was lower in the patients with TC^{-129} than TT^{-129}, both in the colorectal adenocarcinomas ($p=0.040$) and adjacent noncancerous colorectal tissues ($p=0.023$), but their expression ratio was independent of T-129C ($p=0.149$). No such effects was found for G2677A,T and C3435T, but the comparison between variant carriers and non-carriers, *i.e.*, GG^{2677} *vs.* $GT^{2677}+AT^{2677}+TT^{2677}$ (no patients with GA^{2677} and AA^{2677} in this study) and CC^{3435} *vs.* $CT^{3435}+TT^{3435}$, resulted in lower values of the ratio in variant non-carriers; GG^{2677}: 0.18±0.09 *vs.* others^{2677}: 0.99±1.60 ($p=0.048$), CC^{3435}: 0.21±0.07 *vs.* $CT^{3435}+TT^{3435}$: 1.03±1.64 ($p=0.058$).

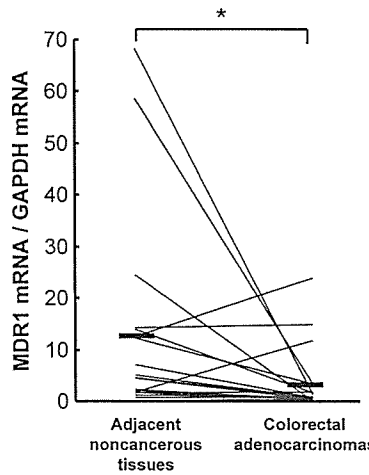

Fig. 1. Relative Concentrations of MDR1 mRNA in Colorectal Adenocarcinomas and Adjacent Noncancerous Colorectal Tissues Obtained from 21 Japanese Patients

Each bar represents the mean value. * $p=0.012$ by Wilcoxon signed-rank test.

DISCUSSION

With a number of clinical investigations, it has been demonstrated that a more preferable clinical response to chemotherapy is found for MDR1-negative tumors than MDR1-positive tumors.[9] Exposure to anticancer drugs sometimes results in the up-regulation of MDR1 in tumor tissues, and therefore in a poorer response when compared with pre-treatment.[9] Since MDR1, originally isolated from resistant tumor cells, appeared unique to sublines displaying an altered permeability to anticancer drugs, the MDR1 expression level-dependent response has been understood, in that MDR1 acts as an efflux pump exporting the anticancer drugs from the inside to the outside of the cells. Recent advances in cell biology have realized the concept of apoptosis to describe cellular events resulting in cell death. Smyth and Johnstone and their co-workers suggested that MDR1 protected cells

Table 1. Effects of Histological Type of Colorectal Adenocarcinomas on MDR1 mRNA Expression in Colorectal Adenocarcinomas and Adjacent Noncancerous Colorectal Tissues Obtained from 21 Japanese Patients

Histological type		Colorectal adenocarcinomas	Adjacent noncancerous colorectal tissues	Ratio[a]
Well-differentiated	5	6.70±6.11	17.73±23.27	1.53±2.59
Moderately-differentiated	12	1.13±1.10	11.97±20.07	0.62±1.10
Others	4	6.50±11.47	10.06±10.82	0.80±0.86
Total	21	3.48±5.97	12.98±18.85[b]	0.87±1.50

The values are the mean±S.D. *a*) Ratio = MDR1 mRNA in colorectal adenocarcinomas/MDR1 mRNA in adjacent noncancerous colorectal tissues. *b*) Higher than colorectal adenocarcinomas with statistical significance of $p=0.012$ by Wilcoxon signed-rank test.

Table 2. Effects of *MDR1* Genotypes of T-129C, G2677A,T and C3435T on MDR1 mRNA Expression in Colorectal Adenocarcinomas and Adjacent Noncancerous Colorectal Tissues Obtained from 21 Japanese Patients

			Colorectal adenocarcinomas		Adjacent noncancerous colorectal tissues		Ratio[a]	
T-129C	TT	18	3.95±6.34		14.68±19.90		0.96±1.61	
	TC	3	0.63±0.20	0.040[b]	2.75±1.76	0.023[b]	0.33±0.30	0.149
G2677A,T	GG	3	2.79±1.98		17.38±15.97		0.18±0.09	
	GT	13	3.36±6.81		10.10±18.53		1.21±1.84	
	AT+TT	5	4.19±5.99	0.949	17.82±23.41	0.694	0.43±0.42	0.448
C3435T	CC	4	1.23±1.41		5.32±4.69		0.21±0.07	
	CT	13	3.12±6.30		17.43±22.84		0.77±1.11	
	TT	4	6.89±7.35	0.403	6.19±5.86	0.407	1.88±2.85	0.283
Total		21	3.48±5.97		12.98±18.85		0.87±1.50	

The values are the mean±S.D. *a*) Ratio=MDR1 mRNA in colorectal adenocarcinomas/MDR1 mRNA in adjacent noncancerous colorectal tissues. *b*) Statistically significant difference was found between TT^{-129} and TC^{-129} ($p<0.05$), but no such difference was found on the genotypes of G2677A,T and C3435T.

against the caspase-dependent apoptosis induced by cytotoxic drugs, Fas ligation, tumor necrosis factor and ultraviolet irradiation.[25,26] We also found that MDR1 expression up-regulated by apoptotic stimuli suppressed caspase-dependent apoptotic signaling, presumably *via* a mitochondrial pathway.[23] Although the role of MDR1 in apoptosis has sometimes been discussed from the viewpoints of the sphingomyelin-ceramide pathway, acidification of the intracellular space, cholesterol esterification and cytokine release from lymphocytes,[11,12] these results are also consistent with the clinical observations that the higher expression of MDR1 results in a poorer response to chemotherapy.

Compared with apoptosis, relatively less information is available concerning the role of MDR1 in differentiation. Recently, we[23] and Goto *et al.*[27] have found that MDR1 mRNA is down-regulated in a human colon carcinoma cell line, Caco-2, prior to the up-regulation of villin mRNA, a marker of differentiation. Here, as shown in Fig. 1, it has been demonstrated that the expression level of MDR1 mRNA was lower in colorectal adenocarcinomas than adjacent noncancerous colorectal tissues ($p=0.012$). Lower levels of MDR1 in cancerous tissues than the adjacent normal tissues were also reported in French patients with renal cell carcinoma[28] and Japanese patients with colorectal carcinoma,[29] but the opposite result was obtained in French patients with advanced breast carcinoma.[30] A lower level of MDR1 mRNA in adenocarcinomas than adjacent noncancerous tissues suggests its down-regulation as a consequence of the malignant transformation of colorectal tissues, possibly with the suppression of differentiation. Potocnik *et al.* indicated a lower MDR1 expression in poorly-differentiated colorectal cancers obtained from Slovenia patients than well-differentiated cancers, with intermediate expression for moderately-differentiated cancers.[31] Poorly-differentiated types are found at 13.8—17.5% in Caucasians,[32,33] being more frequently found than the Japanese; that is, Takeuchi *et al.* reported poorly-, moderately- and well-differentiation types were found at 3.3%, 77.2% and 19.5% in adenocarcinomas, respectively,[16] suggesting a difference in the nature of the cancer between Caucasians and Japanese. In this study, a relatively low expression level of MDR1 mRNA was suggested in moderately-differentiated adenocarcinomas than in those that were well-differentiated, but there was no statistically significant difference ($p=0.111$). To elucidate the association of MDR1 expression with differentiation, a colorectal adenocarcinoma cell line, HCT-15 was treated with two typical differentiating agents, NaB or ATRA,[34—37] and as shown in Fig. 2, it was confirmed that MDR1 mRNA was up-regulated after their treatment. Although these agents do not always induce MDR1, depending on the experimental conditions, including the type of cells,[34—37] MDR1 might be involved in the cellular events resulting in differentiation without malignant transformation. We could not obtain poorly-differentiated adenocarcinomas herein from 21 patients, as expected by the report on frequency by Takeuchi *et al.*,[16] and further investigations with relatively large number of patients should be undertaken, hopefully with poorly-differentiated adenocarcinomas, to confirm the role of MDR1 in differentiation.

In this study, the effects of genetic polymorphisms of *MDR1* on the expression levels of MDR1 mRNA was examined to present alternative marker of prognosis, especially

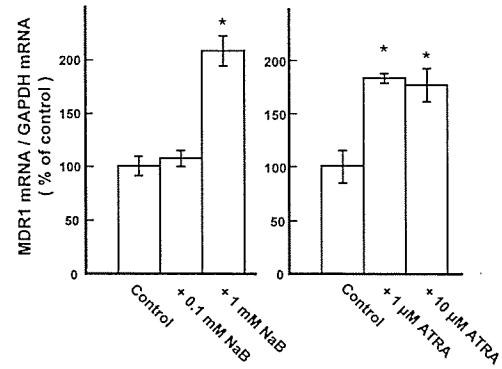

Fig. 2. Effects of Differentiating Agents on MDR1 mRNA Expression in Colorectal Adenocarcinoma Cell Line, HCT-15

(Left) Sodium butyrate (NaB), (Right) all-*trans* retinoic acid (ATRA). The cells were treated with NaB (0.1, 1 mM) or ATRA (1, 10 μM) for 24 h. For control cells, neither of NaB or ATRA was added. Each value represents the mean±S.D. of 5—7 independent experiments. $*p<0.05$, significantly different from the control.

when no extra biopsy samples will be obtained, based on the assumption that a lower expression level of MDR1 mRNA is associated with the suppression of differentiation and the acceleration of proliferation, resulting in the poor prognosis of patients with colorectal adenocarcinoma. A lower expression might induce the malignant transformation of colorectal tissues. Among more than 40 genetic polymorphisms, T-129C, G2677A,T and C3435T are often discussed in terms of their association with the expression,[1—5] and T-129C, but not G2677A,T or C3435T, was found to result in lower expression (Table 2). Such an effect of T-129C was also found in placentas obtained from Japanese people,[38] but we could not find it in duodenum biopsies obtained from healthy Japanese subjects.[21] The ratio of MDR1 mRNA expression in colorectal adenocarcinomas to adjacent noncancerous colorectal tissues was varied with an average of 0.87±1.50, and was independent of T-129C, G2677A,T and C3435T (Table 2). However, the comparison between variant carriers and non-carriers resulted in lower values in non-carriers of G2677A,T and C3435T, *i.e.*, GG^{2677} and CC^{3435}, than corresponding carriers, suggesting that MDR1 mRNA down-regulation after malignant transformation was more likely to be found in the non-carrier patients.

Collectively, the expression level of MDR1 mRNA was lower in colorectal adenocarcinomas than in the adjacent noncancerous colorectal tissues, suggesting its down-regulation as a consequence of the malignant transformation of colorectal tissues, possibly with the suppression of differentiation. A relatively lower expression was suggested in moderately-differentiated colorectal adenocarcinomas than well-differentiated adenocarcinomas. MDR1 mRNA up-regulation was found in HCT-15 cells after treatment with NaB and ATRA, suggesting its involvement in the cellular events resulting in differentiation without malignant transformation. *MDR1* T-129C, but not G2677A,T and C3435T, was associated with the lower expression of MDR1 mRNA both in colorectal adenocarcinomas and adjacent noncancerous colorectal tissues, possibly being useful invasive marker predicting poorly-differentiated colorectal adenocarcinomas, and thereby, the poor prognosis of the patients, especially when no extra biopsy samples will be obtained. Further investigations with relatively large number of patients should be un-

dertaken to confirm these preliminary results.

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REFERENCES

1) Sakaeda T., Nakamura T., Okumura K., *Biol. Pharm. Bull.*, **25**, 1391—1400 (2002).
2) Sakaeda T., Nakamura T., Okumura K., *Pharmacogenomics*, **4**, 397—410 (2003).
3) Sakaeda T., Nakamura T., Okumura K., *Curr. Top. Med. Chem.*, **4**, 1385—1398 (2004).
4) Okamura N., Sakaeda T., Okumura K., *Personalized Med.*, **1**, 85—104 (2004).
5) Sakaeda T., *Drug Metab. Pharmacokinet.*, **20**, 391—414 (2005).
6) Juliano R. L., Ling V., *Biochim. Biophys. Acta*, **455**, 152—162 (1976).
7) Chen C., Chin J. E., Ueda K., Clark D. P., Pastan I., Gottesman M. M., Roninson I. B., *Cell*, **47**, 381—389 (1986).
8) Roninson I. B., Chin J. E., Choi K. G., Gros P., Housman D. E., Fojo A., Shen D. W., Gottesman M. M., Pastan I., *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 4538—4542 (1986).
9) Takara K., Sakaeda T., Okumura K., *Curr. Pharm. Design*, **12**, 273—286 (2006).
10) Thiebaut F., Tsuruo T., Hamada H., Gottesman M. M., Pastan I., Willingham M. C., *Proc. Natl. Acad. Sci. U.S.A.*, **84**, 7735—7738 (1987).
11) Johnstone R. W., Ruefli A. A., Smyth M. J., *Trends Biochem. Sci.*, **25**, 1—6 (2000).
12) Johnstone R. W., Ruefli A. A., Tainton K. M., Smyth M. J., *Leukemia Lymphoma*, **38**, 1—11 (2000).
13) Chaudhary P. M., Roninson I. B., *Cell*, **66**, 85—94 (1991).
14) Ismail T., Hallissey M. T., Fielding J. W., *World J. Surg.*, **19**, 178—183 (1995).
15) Sugao Y., Yao T., Kubo C., Tsuneyoshi M., *Histopathology*, **31**, 123—133 (1997).
16) Takeuchi K., Kuwano H., Tsuzuki Y., Ando T., Sekihara M., Hara T., Asao T., *Hepatogastroenterology*, **51**, 1698—1702 (2004).
17) van Belzen N., Dinjens W. N., Eussen B. H., Bosman F. T., *Histol. Histopathol.*, **13**, 1233—1242 (1998).
18) Sakaeda T., Nakamura T., Horinouchi M., Kakumoto M., Ohmoto N., Sakai T., Morita Y., Tamura T., Aoyama N., Hirai M., Kasuga M., Okumura K., *Pharm. Res.*, **18**, 1400—1404 (2001).
19) Horinouchi M., Sakaeda T., Nakamura T., Morita Y., Tamura T., Aoyama N., Kasuga M., Okumura K., *Pharm. Res.*, **19**, 1581—1585 (2002).
20) Nakamura T., Sakaeda T., Horinouchi M., Tamura T., Aoyama N., Shirakawa T., Matsuo M., Kasuga M., Okumura K., *Clin. Pharmacol. Ther.*, **71**, 297—303 (2002).
21) Moriya Y., Nakamura N., Horinouchi M., Sakaeda T., Tamura T., Aoyama N., Shirakawa T., Gotoh A., Fujimoto S., Matsuo M., Kasuga M., Okumura K., *Biol. Pharm. Bull.*, **25**, 1356—1359 (2002).
22) Nakamura T., Sakaeda T., Ohmoto N., Tamura T., Aoyama N., Shirakawa T., Kamigaki T., Nakamura T., Kim K. I., Kim S. R., Kuroda Y., Matsuo M., Kasuga M., Okumura K., *Drug Metab. Dispos.*, **30**, 4—6 (2002).
23) Sakaeda T., Nakamura T., Hirai M., Kimura T., Wada A., Yagami T., Kobayashi H., Nagata S., Okamura N., Yoshikawa T., Shirakawa T., Gotoh A., Matsuo M., Okumura K., *Pharm. Res.*, **19**, 1323—1329 (2002).
24) Moriya Y., Nakamura T., Okamura N., Sakaeda T., Horinouchi M., Tamura T., Aoyama N., Kasuga M., Okumura K., *Biol. Pharm. Bull.*, **29**, 535—538 (2006).
25) Smyth M. J., Krasovskis E., Sutton V. R., Johnstone R. W., *Proc. Natl. Acad. Sci. U.S.A.*, **95**, 7024—7029 (1998).
26) Johnstone R. W., Cretney E., Smyth M. J., *Blood*, **93**, 1075—1085 (1999).
27) Goto M., Masuda S., Saito H., Inui K., *Biochem. Pharmacol.*, **66**, 163—170 (2003).
28) Oudard S., Levalois C., Andrieu J.-M., Bougaran J., Validire P., Thiounn N., Poupon M.-F., Fourme E., Chevillard S., *Anticancer Res.*, **22**, 121—128 (2002).
29) Hinoshita E., Uchiumi T., Taguchi K., Kinukawa N., Tsuneyoshi M., Maehara Y., Sugimachi K., Kuwano M., *Clin. Cancer Res.*, **6**, 2401—2407 (2000).
30) Arnal M., Franco N., Fargeot P., Riedinger J. M., Brunet-Lecomte P., Lizard-Nacol S., *Breast Cancer Res. Treat.*, **61**, 13—20 (2000).
31) Potocnik U., Ravnik-Glavac M., Golouh R., Glavac D., *J. Med. Genet.*, **39**, 340—346 (2002).
32) Purdie C. A., Piris J., *Histopathology*, **36**, 121—126 (2000).
33) Chung C. K., Zaino R. J., Stryker J. A., *J. Surg. Oncol.*, **21**, 143—148 (1982).
34) Stromskaya T. P., Rybalkina E. Y., Zabotina T. N., Shishkin A. A., Stavrovskaya A. A., *Cancer Cell Int.*, **5**, 15 (2005).
35) Tokura Y., Shikami M., Miwa H., Watarai M., Sugamura K., Wakabayashi M., Satoh A., Imamura A., Mihara H., Katoh Y., Kita K., Nitta M., *Leuk. Res.*, **26**, 29—36 (2002).
36) Frommel T. O., Coon J. S., Tsuruo T., Roninson I. B., *Int. J. Cancer*, **55**, 297—302 (1993).
37) Takeshita A., Shigeno K., Shinjo K., Naito K., Ohnishi K., Hayashi H., Tanimoto M., Ohno R., *Leuk. Lymphoma.*, **42**, 739—746 (2001).
38) Tanabe M., Ieiri I., Nagata N., Inoue K., Ito S., Kanamori Y., Takahashi M., Kurata Y., Kigawa J., Higuchi S., Terakawa N., Otsubo K., *J. Pharmacol. Exp. Ther.*, **297**, 1137—1143 (2001).

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Regular Article

MDR1 Haplotype Frequencies in Japanese and Caucasian, and in Japanese Patients with Colorectal Cancer and Esophageal Cancer

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Summary: The genotype frequencies of *MDR1* T-129C, C1236T, G2677A,T and C3435T SNPs were compared in 154 healthy Japanese and 100 healthy Caucasians to provide basic information on the inter-ethnic differences of pharmacotherapeutic outcome. The variants were found at allelic frequencies of 5.5%, 65.6%, 16.6%, 40.6% and 40.6%, for T-129C, C1236T, G2677A, G2677T and C3435T, respectively, in Japanese, and at 5.1%, 45.9%, 3.6%, 46.4% and 56.6%, respectively, in Caucasians, with a statistically significant difference for C1236T, G2677A,T and C3435T ($p<0.001$). G2677A was about 5-fold more frequent in Japanese than Caucasians. These genotype frequencies were also investigated in 95 Japanese patients with colorectal cancer (CRC) and esophageal squamous cell carcinoma (ESCC), but no significant difference was detected, when compared with healthy Japanese subjects. The haplotype frequency reached a total of about 85% in Japanese with the following 4 major haplotypes; T^{-129}-T^{1236}-T^{2677}-T^{3435} (36.1%), T^{-129}-T^{1236}-G^{2677}-C^{3435} (22.5%), T^{-129}-C^{1236}-G^{2677}-C^{3435} (14.2%) and T^{-129}-C^{1236}-A^{2677}-C^{3435} (13.3%). The second and fourth haplotypes were hardly inferred in Caucasian, whereas T^{-129}-C^{1236}-G^{2677}-T^{3435} (12.8%) was found to be Caucasian-specific. There was a tendency for higher frequencies of the T^{-129}/C^{-129}-C^{1236}-A^{2677}-C^{3435} haplotype in Japanese CRC patients and T^{-129}-T^{1236}-T^{2677}-T^{3435} haplotype in Japanese ESCC patients, compared with that in healthy Japanese subjects.

Key words: MDR1; genotype; haplotype; inter-ethnic difference; colorectal cancer; esophageal cancer

Introduction

Multidrug resistant transporter, MDR1/P-glycoprotein (ABCB1), the gene product of *MDR1*, was originally isolated from cancer cells that had developed resistance to anticancer drugs.[1-8] It has been elucidated that MDR1 is also expressed in normal tissues, including the liver, kidney, small and large intestines, brain, testis, muscle tissue, placenta, and adrenals, and confers an intrinsic resistance by exporting unnecessary and toxic exogenous substances or metabolites out of cells.[1-8] A number of structurally unrelated drugs have been found to be substrates for MDR1, and MDR1 and its related proteins are now recognized as important factors regulating the pharmacokinetics of drugs. Moreover, recent investigations have implicated MDR1 in the

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system regulating cell differentiation, proliferation and survival.

The first systemic screening for *MDR1* genetic polymorphisms was performed by Hoffmeyer *et al.* in 2000, and 15 single nucleotide polymorphisms (SNPs) were identified by analyzing 188 Caucasian individuals.[9] In the ensuring 5 years, several attempts have been made to identify additional SNPs and to examine their association with phenotypes. Recently, more than 40 SNPs were listed in an extensive examination by Kroetz *et al.*[10] Of these SNPs, a silent SNP in exon 26, C3435T, is the best characterized in terms of its association with the expression and/or function in the tissues, and also with pharmacokinetics and pharmacodynamics, however, there are still discrepancies in the results.[1-8]

Based on the assumption that MDR1 plays an important role in the detoxification systems of normal tissues, several studies have focused on the effects of C3435T on susceptibility to a certain class of disease. As for susceptibility to cancer, Siegsmund *et al.*[11] and Jamroziak *et al.*[12] suggested that the T^{3435}-allele is a risk factor for renal epithelial tumors and childhood acute lymphoblastic leukemia (ALL), respectively. C3435T has also reportedly been found more frequently in patients with colon cancer.[13-15] In contrast, Stanulla *et al.* suggested a significant reduction in the risk of relapse in the central nervous system in childhood ALL for patients with the T^{3435}-allele,[16] and Miller *et al.* reported no association with adult glioma.[17] Illmer *et al.* have reported *MDR1* genotype-related susceptibility to acute myeloid leukemia, where the heterozygote for C1236T, G2677T and C3435T was more frequently found among patients.[18] As stated above, there is no consensus on the association of C3435T with susceptibility to cancer, or with pharmacotherapy, indicating the need for a rational explanation and additional clinical investigations.

Herein, the genotype and haplotype frequencies of *MDR1* T-129C, C1236T, G2677A,T and C3435T SNPs were compared in 154 healthy Japanese and 100 healthy Caucasians to provide basic information on the inter-ethnic differences of pharmacotherapeutic outcome between both populations, and moreover were investigated in 95 Japanese patients with colorectal cancer (CRC) and esophageal squamous cell carcinoma (ESCC) to examine their potential as predictors of cancer susceptibility.

Materials and Methods

Subjects: A total of 95 unrelated Japanese patients with CRC (34 males and 14 females) and ESCC (44 males and 3 females) diagnosed at Kobe University Hospital participated in this study. Diagnoses of colorectal adenocarcinoma and esophageal carcinoma were based on clinical, endoscopic, radiologic, and histopathological findings as described previously.[19,20] The average age was 66.4±11.5 (±SD) years old (range, 28–82 years) and 64.8±8.0 years old (range, 48–83 years), respectively. One hundred and fifty-four unrelated healthy Japanese subjects (47 males and 107 females) aged 26.3±7.5 (range, 21–57) years were also enrolled and served as the healthy Japanese subjects. Written informed consent was obtained from all patients at the beginning of this study. The protocol for this study was approved by the Institutional Review Board of Kobe University Hospital, Japan. The data for MDR1 genotypes in 100 healthy Caucasians were quoted from a study which was performed as the Pharmacogenetics of Membrane Transporter Project at the University of California, San Francisco in the United States.[10]

Isolation of genomic DNA: Peripheral blood (2.0 mL) was drawn from the subject into a sampling tube containing EDTA-2Na (3.0 mg), and genomic DNA was extracted from 0.2 mL of whole blood using a QIAamp DNA Blood mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions.

MDR1 genotyping of T-129C, C1236T, G2677A,T and C3435T: The genetic polymorphisms T-129C, G2677A,T and C3435T were determined using the TaqMan® MGB probe and primer as reported previously.[21] To determine C1236T, the following probe and primers were designed using the software Assays-by-DesignSM (Applied Biosystems, Foster, USA): the C^{1236}-allele probe, 5′-CAG GTT CAG gCC CTT-3′; the T^{1236}-allele probe, 5′-TTC AGG TTC AGa CCC TT-3′; the forward primer, 5′-CAC CGT CTG CCC ACT CT-3′; and the reverse primer, 5′-GTG TCT GTG AAT TGC CTT GAA GTT T-3′. Lower-case font represents the SNP. As a reporter at the 5′ end of the TaqMan® MGB probe, VIC® was used for the C^{1236}-allele and 6-carboxyfluorescein (FAM) was used for the T^{1236}-allele. All TaqMan® MGB probes and primers used in this study were synthesized by Applied Biosystems Japan, Ltd. (Tokyo, Japan).

Haplotype analysis: The *MDR1* haplotypes were statistically inferred using an algorithm based on Bayesian inference, PHASE version 2.0.2 (http://www.stat.Washington.edu/stephens/) with a fair degree of precision.[10,22,23] PHASE calls were made separately for each ethnic and disease group. Haplotypes were inferred by running PHASE a total of 10 times, and relative standard deviation of their frequencies was 5% or less of the mean value. The estimated haplotype frequencies were expressed as the average population haplotype frequencies for the whole sample (referred to as the "Population haplotype") as well as the practical haplotype frequencies based on the most likely inferred pairs of haplotypes identified at least 8 of 10 times for each individual (referred to as the "Practical haplo-

Table 1. Allelic and genotype frequencies of *MDR1* T-129C, C1236T, G2677A,T and C3435T in Japanese and Caucasians, and in Japanese patients with colorectal cancer and esophageal cancer.

SNP	Subject[a]	N	Allele				Genotype						
T-129C			T	C		*P*-value[b]	TT	TC	CC				*P*-value[b]
	Japanese Healthy	154	291 94.5%	17 5.5%			139 90.3%	13 8.4%	2 1.3%				
	CRC	48	89 92.7%	7 7.3%		0.620	41 85.4%	7 14.6%	0 0.0%				—
	ESCC	47	88 93.6%	6 6.4%		0.800	41 87.2%	6 12.8%	0 0.0%				—
	Caucasian Healthy[c]	99	188 94.9%	10 5.1%		1.000	89 89.9%	10 10.1%	0 0.0%				—
C1236T			C	T			CC	CT	TT				
	Japanese Healthy	154	106 34.4%	202 65.6%			17 11.0%	72 46.8%	65 42.2%				
	CRC	48	38 39.6%	58 60.4%		0.393	7 14.6%	24 50.0%	17 35.4%				0.618
	ESCC	47	39 41.5%	55 58.5%		0.222	6 12.8%	27 57.4%	14 29.8%				0.299
	Caucasian Healthy[c]	97	105 54.1%	89 45.9%		<0.001	29 29.9%	47 48.5%	21 21.6%				<0.001
G2677A,T			G	A	T		GG	GA	GT	AA	AT	TT	
	Japanese Healthy	154	132 42.9%	51 16.6%	125 40.6%		30 19.5%	23 14.9%	49 31.8%	4 2.6%	20 13.0%	28 18.2%	
	CRC	48	36 37.5%	20 20.8%	40 41.7%	0.528	8 16.7%	5 10.4%	15 31.3%	2 4.2%	11 22.9%	7 14.6%	—
	ESCC	47	39 41.5%	14 14.9%	41 43.6%	0.867	8 17.0%	5 10.6%	18 38.3%	0 0.0%	9 19.1%	7 14.9%	—
	Caucasian Healthy[c]	98	98 50.0%	7 3.6%	91 46.4%	<0.001	27 27.6%	3 3.1%	41 41.8%	1 1.0%	2 2.0%	24 24.5%	—
C3435T			C	T			CC	CT	TT				
	Japanese Healthy	154	183 59.4%	125 40.6%			55 35.7%	73 47.4%	26 16.9%				
	CRC	48	56 58.3%	40 41.7%		0.905	14 29.2%	28 58.3%	6 12.5%				0.411
	ESCC	47	50 53.2%	44 46.8%		0.286	11 23.4%	28 59.6%	8 17.0%				0.255
	Caucasian Healthy[c]	99	86 43.4%	112 56.6%		<0.001	21 21.2%	44 44.4%	34 34.3%				0.003

[a]CRC: Colorectal cancer, ESCC: Esophageal squamous cell carcinoma
[b]Allelic and Genotype frequency comparisons with Japanese healthy subjects (Fisher's exact test).
[c]Data from the study reported by Kroetz *et al.*[10]

type'').

Statistical analysis: Differences in genotype and allelic frequencies between healthy Japanese and Caucasian subjects and between the healthy subjects and patients in Japan were examined using Fisher's exact statistical tests. P values of less than 0.05 were considered significant.

Results

Table 1 lists the *MDR1* allelic and genotype frequencies determined in healthy Japanese and Caucasian subjects, and in Japanese patients with CRC and ESCC. In healthy Japanese subjects, the variants were found at allelic frequencies of 5.5%, 65.6%, 16.6%, 40.6% and 40.6%, for T-129C, C1236T, G2677A, G2677T and

Table 2. Estimated frequencies of *MDR1* haplotypes in Japanese and Caucasians, and in Japanese patients with colorectal cancer and esophageal cancer.

Position				Nomenclature[a]	Estimated Frequency (%)[b]			
					Japanese			Caucasian[c,d] Healthy
−129	1236	2677	3435		Healthy N=154	CRC N=48	ESCC N=47	N=95
T	T	T	T	**13/*13A/*14/*14A/*16*	36.1 (36.4)	37.1 (37.5)	42.3 (42.6)	40.0 (41.1)
T	T	G	C	**11/*11A/*11B/*19*	22.5 (22.4)	17.8 (16.7)	15.3 (14.9)	1.0 (1.1)
T	C	G	C	**1/*8/*8A/*9/*9A/*9B/*9C/ *10/*20/*21/*21A/*21B/*21E/ *22/*22A/*23/*23A/*25/*26/ *26A/*26B/*26D/*27/*28/ *29/*30/*31/*32*	14.2 (14.6)	15.7 (16.7)	19.5 (20.2)	32.5 (32.6)
T	C	A	C	**24/*24A*	13.3 (13.0)	14.6 (14.6)	10.8 (10.6)	1.6 (1.6)
T	T	T	C	**15/*15A*	4.0 (3.9)	3.2 (3.1)	0.6 (1.1)	3.1 (2.6)
C	C	A	C	**4A*	3.1 (3.6)	6.1 (6.3)	3.9 (4.3)	<0.1 (−)
T	T	G	T	**12/*12A/*18/*19A*	2.5 (2.6)	1.9 (3.1)	<0.1 (−)	0.5 (0.5)
C	C	G	C	**3/*4/*4B/*5/*5A/*6/ *6A/*7*	1.9 (1.6)	0.5 (1.0)	2.4 (2.1)	3.7 (4.2)
T	C	G	T	**2/*2A/*17/*21C*	1.5 (1.3)	1.0 (−)	4.2 (4.3)	12.8 (12.1)
C	T	G	C	*not assigned*	0.4 (0.3)	0.2 (−)	<0.1 (−)	<0.1 (−)
T	C	T	T	**17A*	0.3 (0.3)	1.2 (1.0)	<0.1 (−)	1.0 (1.1)
C	C	T	T	*not assigned*	0.1 (−)	<0.1 (−)	<0.1 (−)	<0.1 (−)
T	C	T	C	**21D/*26C*	<0.1 (−)	<0.1 (−)	0.5 (−)	1.7 (1.6)
T	C	A	T	**2B*	<0.1 (−)	<0.1 (−)	<0.1 (−)	1.0 (1.1)
C	C	G	T	*not assigned*	<0.1 (−)	0.3 (−)	<0.1 (−)	0.5 (0.5)
C	T	G	T	**12B*	<0.1 (−)	0.1 (−)	<0.1 (−)	<0.1 (−)
C	T	A	T	*not assigned*	<0.1 (−)	<0.1 (−)	0.1 (−)	<0.1 (−)
C	T	T	T	*not assigned*	<0.1 (−)	<0.1 (−)	<0.1 (−)	0.5 (−)

[a]Allele assigned by Kroetz *et al.*[10] based on 64 distinct haplotypes obtained for 28 variant sites in a total of 494 subjects. Sixty-three were inferred for either of the groups, but *MDR1*15B* (C^{-129}-T^{1236}-T^{2677}-C^{3435}) was not detected.
[b]"Population haplotype" frequencies are presented with "Practical haplotype" frequencies in parentheses. —: Not inferred.
[c]Haplotype frequencies were calculated in 95 healthy Caucasian subjects.
[d]Data from the study reported by Kroetz *et al.*[10]

C3435T respectively. For healthy Caucasians, they were found at 5.1%, 45.9%, 3.6%, 46.4% and 56.6%, respectively, with a statistically significant difference for C1236T, G2677A,T and C3435T ($p<0.001$). It is noted that G2677A was about 5-fold more frequently found in Japanese than Caucasian. Statistically significant differences were also detected for their genotypes (**Table 1**). The variants were found at frequencies of

7.3%, 60.4%, 20.8%, 41.7% and 41.7% for 48 Japanese CRC patients and 6.4%, 58.5%, 14.9%, 43.6% and 46.8% for 47 Japanese ESCC patients, respectively. No significant difference was detected for allelic and genotype frequencies between healthy Japanese subjects and either group of cancer patients.

Table 2 shows the estimated frequencies of *MDR1* haplotypes in healthy Japanese and Caucasian subjects, and in Japanese patients with CRC and ESCC. No difference in frequency was found between the "Population haplotype" and "Practical haplotype". In healthy Japanese subjects, 12 haplotypes were statistically inferred with a frequency of more than 0.1% at the population base. With the major 4 haplotypes, the frequency reached a total of about 85%; T^{-129}-T^{1236}-T^{2677}-T^{3435} (36.1%), T^{-129}-T^{1236}-G^{2677}-C^{3435} (22.5%), T^{-129}-C^{1236}-G^{2677}-C^{3435} (14.2%) and T^{-129}-C^{1236}-A^{2677}-C^{3435} (13.3%). T^{-129}-T^{1236}-G^{2677}-C^{3435} (1.0%) and T^{-129}-C^{1236}-A^{2677}-C^{3435} (1.6%) were rare, and 3 of 12 haplotypes found in Japanese were not statistically inferred with a frequency of more than 0.1% in Caucasians, whereas T^{-129}-C^{1236}-G^{2677}-T^{3435} (12.8%) was Caucasian-specific. There was no significant difference in the estimated frequencies of *MDR1* haplotypes between healthy Japanese subjects and Japanese CRC or ESCC patients, although there was a tendency for higher frequencies of the T^{-129}/C^{-129}-C^{1236}-A^{2677}-C^{3435} haplotype in CRC patients and T^{-129}-T^{1236}-T^{2677}-T^{3435} haplotype in ESCC patients, compared with that in healthy Japanese subjects (**Table 1**).

Discussion

In this examination, the frequency of variants T-129C, C1236T, G2677A, G2677T and C3435T in healthy Japanese subjects was 5.5%, 65.6%, 16.6%, 40.6% and 40.6%, respectively (**Table 1**). These results are consistent with other studies on Japanese and our previous report with a smaller population, i.e., 6.0–9.3% for T-129C,[21,24-26] 55.5–61.5% for C1236T,[24,25] 16.9–20.5% for G2677A,[21,24-26] 35.5–40.8% for G2677T,[21,24-26] and 38.5–44.1% for C3435T.[21,24-27] In Caucasians, the allelic frequencies were found to be 5.1%, 45.9%, 3.6%, 46.4% and 56.6%, respectively, again consistent with previous reports, i.e., 5.1–5.9% for T-129C,[9,10] 37.8–45.9% for C1236T,[9,10,28,29] 1.9–3.6% for G2677A,[10,28,29] 41.6–46.4% for G2677T,[10,28,29] and 48.0–56.1% for C3435T.[9,10,28,29] Inter-ethnic differences in frequencies have been studied extensively for C3435T among SNPs ever since the report by Hoffmeyer *et al.* on the effects of *MDR1* genotype showed the association of C3435T with the expression of MDR1 in the duodenum, and thereby with the pharmacokinetics of digoxin, a typical MDR1 substrate, after oral administration.[9] In the past 5 years, a number of clinical studies have been performed to replicate these findings; however, there are still discrepancies, suggesting the effects of other SNPs.[1-8] The allelic and genotype frequencies for T-129C, C1236T and G2677A,T presented herein might be useful for explaining the differences with the findings of Hoffmeyer *et al.*[9]

MDR1 is understood to play an important role in the detoxification systems of normal tissues, and C3435T has been evaluated in terms of disease susceptibility. Several reports have suggested that this SNP is a risk factor for cancer, including colorectal cancer,[11-15] although this is not always the case.[16,17] Here, it was found that C3435T was not associated with CRC or ESCC in Japanese. Additionally, the effects of 3 other SNPs were evaluated. Although there was a tendency for a higher frequency of the A^{2677}-allele in the CRC patients, the T^{3435}-allele in the ESCC patients, and TC^{-129}, CT^{1236}, AT^{2677} and CT^{3435} genotypes in the CRC and ESCC patients, compared to healthy Japanese subjects, the study was not sufficiently powered to reach statistical significance (**Table 1**). In Japan, the incidence of CRC and ESCC is increasing in Japan,[30] and therefore further studies are needed to examine whether environmental factors, unexamined *MDR1* genotypes, and/or genes other than *MDR1* gene, were more predominant for the development of CRC and ESCC.

To explain the diversity of the results of C3435T, several reports suggested the importance of linkage disequilibrium of C3435T with C1236T and G2677T, the latter resulting in Ala893Ser, that is, C3435T may not itself be causal but rather may be linked with the causal polymorphisms.[1-8] Johne *et al.* defined 4 haplotypes; 11, 12, 21 and 22, and 9 genotypes; 00, 01, 02, 10, 11, 12, 02, 21 and 22, based on G2677T and C3435T,[31] where the haplotype coding is as follows; 1: identical to the reference sequence (G^{2677}/C^{3435}); 2: different from it (T^{2677}/T^{3435}), and therefore the genotype coding is as follows; 0: homozygous for nucleotides identical to the reference sequence for the position on both chromosomes; 1: heterozygous; 2: homozygous for nucleotides different from the reference sequence for the position on both chromosomes. For genotype 11, 11/22, not 12/21, is selected based on the frequency in Caucasians. This assignment is often used to check the importance of haplotype analyses,[1-8] but it is noted that a variant A^{2677}-allele was preferentially found in Japanese as shown in **Table 1**. This allele has been shown to be important for the pharmacokinetics of the H_1-antihistamine, fexofenadine,[32] and thus an analysis based on Johne's assignment is insufficient for Japanese. Haplotype may often provide more useful information than genotype about inter-individual and inter-ethnic differences of pharmacokinetics and pharmacodynamics.[33] Kim *et al.*,[29] Sai *et al.*,[24] and Tang *et al.*[34] have defined 11 haplotypes, *MDR1*1* to **11*, and their subtypes based on C1236T, G2677A,T and C3435T, but Saito

et al. have suggested the importance of T-129C.[21] Kroetz *et al.* defined 32 haplotypes, *MDR1*1* to *MDR1*32*, and their subtypes (a total of 64 haplotypes).[10] These assignments should be justified by genotype-phenotype correlation studies. As shown in **Table 2**, there is a considerable difference in the frequencies of haplotype including A^{2677} polymorphism between Japanese and Caucasian. This might contribute in part to the inter-ethnic discrepancies of the results on C3435T.

Estimates of *MDR1* haplotype frequencies showed that 3 of 12 haplotypes found in Japanese were hardly inferred in Caucasians, whereas T^{-129}-C^{1236}-G^{2677}-T^{3435} was Caucasian-specific. A tendency for higher frequencies of the T^{-129}/C^{-129}-C^{1236}-A^{2677}-C^{3435} haplotype in the CRC patients and the T^{-129}-T^{1236}-T^{2677}-T^{3435} haplotype in the ESCC patients was also observed in comparison with that in the healthy Japanese subjects. These results suggested that *MDR1* haplotypes based on 4 sites, −129, 1236, 2677 and 3435, may be able to be used to characterize ESCC and CRC patients in the Japanese population, and future large scale studies are warranted to appropriately investigate this possibility.

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References

1) Sakaeda, T., Nakamura, T. and Okumura, K.: *MDR1* genotype-related pharmacokinetics and pharmacodynamics. *Biol. Pharm. Bull.*, **25**: 1391–1400 (2002).
2) Sakaeda, T., Nakamura, T. and Okumura, K.: Pharmacogenetics of *MDR1* and its impact on the pharmacokinetics and pharmacodynamics of drugs. *Pharmacogenomics*, **4**: 397–410 (2003).
3) Marzolini, C., Paus, E., Buclin, T. and Kim, R. B.: Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. *Clin. Pharmacol. Ther.*, **75**: 13–33 (2004).
4) Woodahl, E. L. and Ho, R. J.: The role of MDR1 genetic polymorphisms in interindividual variability in P-glycoprotein expression and function. *Curr. Drug Metab.*, **5**: 11–19 (2004).
5) Pauli-Magnus, C. and Kroetz, D. L.: Functional implications of genetic polymorphisms in the multidrug resistance gene MDR1 (ABCB1). *Pharm. Res.*, **21**: 904–913 (2004).
6) Okamura, N., Sakaeda, T. and Okumura, K.: Pharmacogenomics of *MDR* and *MRP* families. *Personalized Med.*, **1**: 85–104 (2004).
7) Sakaeda, T., Nakamura, T. and Okumura, K.: Pharmacogenetics of drug transporters and its impact on the pharmacotherapy. *Curr. Top. Med. Chem.*, **4**: 1385–1398 (2004).
8) Ieiri, I., Takane, H. and Otsubo, K.: The MDR1 (ABCB1) gene polymorphism and its clinical implications. *Clin. Pharmacokinet.*, **43**: 553–576 (2004).
9) Hoffmeyer, S., Burk, O., von Richter, O., Arnold, H. P., Brockmoller, J., Johne, A., Cascorbi, I., Gerloff, T., Roots, I., Eichelbaum, M. and Brinkmann, U.: Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc. Natl. Acad. Sci. USA*, **97**: 3473–3478 (2000).
10) Kroetz, D. L., Pauli-Magnus, C., Hodges, L. M., Huang, C. C., Kawamoto, M., Johns, S. J., Stryke, D., Ferrin, T. E., DeYoung, J., Taylor, T., Carlson, E. J., Herskowitz, I., Giacomini, K. M. and Clark, A. G.: Sequence diversity and haplotype structure in the human ABCB1 (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics*, **13**: 481–494 (2003).
11) Siegsmund, M., Brinkmann, U., Schaffeler, E., Weirich, G., Schwab, M., Eichelbaum, Fritz, P., Burk, O., Decker, J., Alken, P., Rothenpieler, U., Kerb, R., Hoffmeyer, S. and Brauch, H.: Association of the P-glycoprotein transporter MDR1 (C3435T) polymorphism with the susceptibility to renal epithelial tumors. *J. Am. Soc. Nephrol.*, **13**: 1847–1854 (2002).
12) Jamroziak, K., Mlynarski, W., Balcercza, E., Mistygacz, M., Trelinska, J., Mirowski, M., Bodalski, J. and Robak, T.: Functional C3435T polymorphism of MDR1 gene: an impact on genetic susceptibility and clinical outcome of childhood acute lymphoblastic leukemia. *Eur. J. Haematol.*, **72**: 314–321 (2004).
13) Potocnik, U., Ravnik-Glavac, M., Golouh, R. and Glavac, D.: Naturally occurring mutations and functional polymorphisms in multidrug resistance 1 gene: correlation with microsatellite instability and lymphoid infiltration in colorectal cancers. *J. Med. Genet.*, **39**: 340–346 (2002).
14) Humeny, A., Rodel, F., Rodel, C., Sauer, R., Fuzesi, L., Becker, C. and Efferth, T.: MDR1 single nucleotide polymorphism C3435T in normal colorectal tissue and colorectal carcinomas detected by MALDI-TOF mass spectrometry. *Anticancer Res.*, **23**: 2735–2740 (2003).
15) Kurzawski, M., Drozdzik, M., Suchy, J., Kurzawski, G., Bialecka, M., Gornik, W. and Lubinski, J.: Polymorphism in the P-glycoprotein drug transporter MDR1 gene in colon cancer patients. *Eur. J. Clin. Pharmacol.*, **61**: 389–394 (2005).
16) Stanulla, M., Schaffeler, E., Arens, S., Rathmann, A., Schrauder, A., Welte, K., Eichelbaum, M., Zanger, U. M., Schrappe, M. and Schwab, M.: GSTP1 and MDR1 genotypes and central nervous system relapse in childhood acute lymphoblastic leukemia. *Int. J. Hematol.*, **81**: 39–44 (2005).
17) Miller, K. L., Kelsey, K. T., Wiencke, J. K., Moghadassi, M., Miike, R., Liu, M. and Wrensch, M.: The C3435T polymorphism of MDR1 and susceptibility to adult glioma. *Neuroepidemiology.*, **25**: 85–90 (2005).
18) Illmer, T., Schuler, U. S., Thiede, C., Schwarz, U. I., Kim, R. B., Gotthard, S., Freund, D., Schakel, U.,

Ehninger, G. and Schaich, M.: MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res.*, **62**: 4955–4962 (2002).
19) Nakahara, T., Sakaeda, T., Nakamura, T., Tamura T., Nishioka, C., Aoyama, N., Okamura, N., Shirakawa, T., Gotoh, A., Kamigaki, T., Ohno, M., Kuroda, Y., Matsuo, M., Kasuga, M. and Okumura, K.: Chemosensitivity assessed by collagen gel droplet embedded culture drug sensitivity test, and MDR1, MRP1, and MRP2 mRNA expression in human colorectal adenocarcinomas. *Pharm. Res.*, **21**: 406–412 (2004).
20) Miki, I., Tamura, T., Nakamura, T., Makimoto, H., Hamana, N., Uchiyama, H., Shirasaka, D., Morita, Y., Yamada, H., Aoyama, N., Sakaeda, T., Okumura, K. and Kasuga M.: Circadian variability of pharmacokinetics of 5-fluorouracil and CLOCK T3111C genetic polymorphism in patients with esophageal carcinoma. *Ther. Drug. Monit.*, **27**: 369–374 (2005).
21) Saito, K., Miyake, S., Moriya, H., Yamazaki, M., Itoh, F., Imai, K., Kurosawa, N., Owada, E. and Miyamoto, A.: Detection of the four sequence variants of MDR1 gene using TaqMan MGB probe based real-time PCR and haplotype analysis in healthy Japanese subjects. *Clin. Biochem.*, **36**: 511–518 (2003).
22) Stephens, M., Smith, N. J. and Donnelly. P.: A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.*, **68**: 978–989 (2001).
23) Niu, T.: Algorithms for inferring haplotypes. *Genet. Epidemiol.*, **27**: 334–347 (2004).
24) Sai, K., Kaniwa, N., Itoda, M., Saito, Y., Hasegawa, R., Komamura, K., Ueno, K., Kamakura, S., Kitakaze, M., Shirao, K., Minami, H., Ohtsu, A., Yoshida, T., Saijo, N., Kitamura, Y., Kamatani, N., Ozawa, S. and Sawada, J.: Haplotype analysis of ABCB1/MDR1 blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan. *Pharmacogenetics*, **13**: 741–757 (2003).
25) Tanabe, M., Ieiri, I., Nagata, N., Inoue, K., Ito, S., Kanamori, Y., Takahashi, M., Kurata, Y., Kigawa, J., Higuchi, S., Terakawa, N. and Otsubo, K.: Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J. Pharmacol. Exp. Ther.*, **297**: 1137–1143 (2001).
26) Horinouchi, M., Sakaeda, T., Nakamura, T., Morita, Y., Tamura, T., Aoyama, N., Kasuga, M. and Okumura, K.: Significant genetic linkage of *MDR1* polymorphisms at positions 3435 and 2677: Functional relevance to pharmacokinetics of digoxin. *Pharm. Res.*, **19**: 1581–1585 (2002).
27) Sakaeda, T., Nakamura, T., Horinouchi, M., Kakumoto, M., Ohmoto, N., Sakai, T., Morita, Y., Tamura, T., Aoyama, N., Hirai, M., Kasuga, M. and Okumura, K.: MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm. Res.*, **18**: 1400–1404 (2001).
28) Cascorbi, I., Gerloff, T., Johne, A., Meisel, C., Hoffmeyer, S., Schwab, M., Schaeffeler, E., Eichelbaum, M., Brinkmann, U. and Roots, I.: Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter *MDR1* gene in white subjects. *Clin. Pharmacol. Ther.*, **69**: 169–174 (2001). (Erratum in: *Clin Pharmacol Ther.*, **75**: 124 (2004).)
29) Kim, R. B., Leake, B. F., Choo, E. F., Dresser, G. K., Kubba, S. V., Schwarz, U. I., Taylor, A., Xie, H. G., McKinsey, J., Zhou, S., Lan, L. B., Schuetz, J. D., Schuetz, E. G. and Wilkinson, G. R.: Identification of functionally variant *MDR1* alleles among European Americans and African Americans. *Clin. Pharmacol. Ther.*, **70**: 189–199 (2001).
30) Yiu, H. Y., Whittemore, A. S. and Shibata, A.: Increasing colorectal cancer incidence rates in *Japan. Int. J. Cancer.*, **109**: 777–781 (2004).
31) Johne, A., Kopke, K., Gerloff, T., Mai, I., Rietbrock, S., Meisel, C., Hoffmeyer, S., Kerb, R., Fromm, M. F., Brinkmann, U., Eichelbaum, M., Brockmoller, J., Cascorbi, I. and Roots, I.: Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin. Pharmacol. Ther.*, **72**: 584–594 (2002).
32) Yi, S. Y., Hong, K. S., Lim, H. S., Chung, J. Y., Oh, D. S., Kim, J. R., Jung, H. R., Cho, J. Y., Yu, K. S., Jang, I. J. and Shin, S. G.: A variant 2677A allele of the MDR1 gene affects fexofenadine disposition. *Clin.. Pharmacol. Ther.*, **76**: 418–427 (2004).
33) Colombo, S., Soranzo, N., Rotger, M., Sprenger, R., Bleiber, G., Furrer, H., Buclin, T., Goldstein, D., Decosterd, L. and Telenti, A.: Influence of ABCB1, ABCC1, ABCC2, and ABCG2 haplotypes on the cellular exposure of nelfinavir *in vivo*. *Pharmacogenet. Genom.*, **15**: 599–608 (2005).
34) Tang, K., Ngoi, S. M., Gwee, P. C., Chua, J. M., Lee, J. E., Chong, S. S. and Lee, G. C.: Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics.*, **12**: 437–450 (2002).

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GASTROENTEROLOGY

HLA-DQB1 locus and gastric cancer in *Helicobacter pylori* infection

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Abstract

Background and Aims: It has been suggested that the incidence of digestive diseases associated with *Helicobacter pylori* is influenced by the strain diversity of *H. pylori*, factors involving the host or environment, and the duration of infection. The authors have previously reported that human leukocyte antigen (HLA)-DQB1*0401 plays an important role in the development of atrophic gastritis in *H. pylori* infected patients. The aim of the present study was to investigate the relationship between HLA-DQB1 genotype and cancer development.

Methods: HLA-DQB1 genotyping was performed by the PCR-RFLP method on 122 *H. pylori*-infected non-ulcer dyspepsia (NUD) patients, 53 gastric cancer patients and 28 uninfected controls. To reliably estimate the grade of atrophic gastritis, histological evaluation was performed.

Results: The allele frequency of DQB1*0401 was significantly higher in intestinal type cancer patients compared with age- and sex-matched *H. pylori*-infected NUD patients. There was no significant difference in the mean atrophic scores of the biopsy samples from the lesser curvature of the mid-corpus between these groups.

Conclusions: HLA-DQB1*0401 is a useful marker for determining susceptibility to intestinal type gastric cancer.

Key words: gastric cancer, *Helicobacter pylori*, HLA-DQB1.

INTRODUCTION

Helicobacter pylori is known to be involved in peptic ulcer[1] and the development of atrophic gastritis,[2] and was suggested to be a risk factor for gastric cancer.[3–6] Although more than 50% of the Japanese population is infected with *H. pylori*, only a small percentage of the infected population suffers from peptic ulcers or gastric cancer. This discrepancy may be due to the strain diversity of *H. pylori*, factors involving the host or environment, and the duration of infection. Little is known about the relationship between these factors and *H. pylori*-related diseases. Our previous study revealed that human leukocyte antigen (HLA)-DQB1*0401 plays an important role in the development of atrophic gastritis in *H. pylori* infected patients.[7] Other previous investigations have linked specific HLA class II alleles to cancer development.[8–11]

Human leukocyte antigen class II molecules are α–β heterodimeric membrane glycoproteins that are expressed on the surface of antigen-presenting cells such as macrophages, dendritic cells and B lymphocytes.[12] Helper T cells can only recognize peptides, derived from extracellular antigens, that are associated with HLA class II molecules. The interaction of T cell receptors, peptides and HLA class II molecules determines T cell activation and an immune response to antigens.[13] HLA polymorphism is responsible for variations in the immune response of different individuals to different antigens, and contributes to the susceptibility or resistance to infectious and autoimmune diseases.[14–18] HLA class II genes are

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