Correlation of Induction of ATP Binding Cassette Transporter A5 (ABCA5) and ABCB1 mRNAs with Differentiation State of Human Colon Tumor

Sumio OHTSUKI,[a,b,c] Mayu KAMOI,[a] Yuki WATANABE,[a] Hiroya SUZUKI,[a] Satoko HORI,[a,b,c] and Tetsuya TERASAKI*,[a,b,c]

[a] *Department of Molecular Biopharmacy and Genetics, Graduate School of Pharmaceutical Sciences, Tohoku University;* [b] *New Industry Creation Hatchery Center, Tohoku University; Aoba, Aramaki, Aoba-ku, Sendai 980–8578, Japan: and* [c] *CREST and SORST of the Japan Science and Technology Agency (JST); Japan.*

Received March 2, 2007; accepted March 19, 2007

The ATP binding cassette transporter subtype A5 (ABCA5)-like transporters ABCA5, ABCA6, ABCA8, ABCA9 and ABCA10 form a unique gene cluster within the ABC transporter superfamily, though their function is still poorly understood. The purpose of this study is to examine whether ABCA5-like transporters may play a role in tumor development by measuring their mRNA levels in human tissues and tumors. Intense mRNA expression of human ABCA5-like transporters was detected in the brain. ABCA5 and ABCA8 mRNAs were detected in spleen, testis and ovary. ABCA5 mRNA was also detected in liver and pancreas. ABCA6 mRNA was detected in lung and liver, and ABCA8 was detected in lung. ABCA6, ABCA7 and ABCA8 mRNAs were not detected in any tumors, but weak mRNA expression of ABCA10 was detected in all tumors examined. ABCA5 mRNA was detected in poorly differentiated colon adenocarcinoma (GI-112) and undifferentiated ovarian carcinoma (GI-102), but not in normal colon. ABCB1 mRNA was also detected in GI-112, while ABCC1 and ABCA2 mRNAs were not. In contrast, ABCC1 and ABCA2 mRNAs, but not ABCA5 or ABCB1 mRNA, were detected in well differentiated colon adenocarcinoma (CX-1). Thus, induction of ABCA5, together with ABCB1, appears to be correlated with the differentiation state of human colon tumors, and may have a role in tumor development.

Key words ATP binding cassette (ABC) transporter; ABC transporter A5 (ABCA5); tumor; mRNA expression; colon adenocarcinoma

Members of the ATP binding cassette (ABC) transporter family transport various substrates coupled with hydrolysis of ATP. The human ABC transporter superfamily consists of 49 subtypes, some of which function as drug efflux transporters (ABCB1, ABCC subfamily and ABCG2) or sterol transporters (ABCA1, ABCA7, ABCG1, ABCG5 and ABCG8).[1,2] However, the role of the ABCA5-like transporters remains largely unknown.

The ABCA family forms the second largest gene family, consisting of 12 subtypes, after the ABCC family. Evolutional analysis has revealed a gene cluster encoding ABCA5, ABCA6, ABCA8, ABCA9 and ABCA10, which are known as the ABCA5-like transporters.[3] Interestingly, although these five ABCA transporters form a unique cluster on human Chr17q24, most other ABC transporter genes are dispersed in the mammalian genome.[3] Among the ABCA5-like transporters, ABCA8 expressed in oocytes was shown to exhibit ATP-dependent transport of estradiol-β-glucuronide.[4] In the testis, ABCA5 is localized in Leydig cells, which form the blood-testis barrier, and ABCA5 knockout mice develop an enlarged heart, injured liver and decreased plasma levels of thyroid hormones.[5,6] These observations imply the physiological importance of ABCA5-like transporters.

Since ABCA8 has been reported to act as a transporter,[4] it is possible that ABCA5-like transporters have functions related to multi-drug resistance in tumor cells. The purpose of this study is, therefore, to investigate mRNA expression of ABCA5-like transporters in human tissues and tumors, in order to establish whether there is a relationship between ABCA5-like transporters and tumor development.

MATERIALS AND METHODS

cDNA Samples Human tissue normalized first-strand cDNAs were purchased as MTC Multiple Tissue cDNA Panel I and II from Clontech (Palo Alto, CA, U.S.A.). Human tumor normalized first-strand cDNAs were purchased as MTC Multiple Tumor cDNA Panel from Clontech. The first-strand cDNA was reverse-transcribed from poly-A RNA purified from each tissue or tumor. MTC Panel I contains cDNA preparations from heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas pooled from 2—15 male/female Caucasians. MTC Panel II contains cDNA preparations from spleen, thymus, prostate, testis, ovary, small intestine without mucosal lining, and colon pooled from 7—45 male/female Caucasians, and peripheral blood leukocytes pooled from male/female Caucasians negative for HIV-1, HIV-2, hepatitis B and syphilis.

MTC Tumor Panel contains cDNA preparations from breast carcinoma (GI-101), lung carcinoma (LX-1), colon adenocarcinoma (CX-1), lung carcinoma (GI-117), prostate adenocarcinoma (PC3), colon adenocarcinoma (GI-112), ovarian carcinoma (GI-102) and pancreatic adenocarcinoma (GI-103). GI-101 is a poorly differentiated mammary carcinoma isolated from recurrent ductal carcinoma.[7] LX-1 is a poorly differentiated carcinomal surgical explant from a metastasis in a 48-year-old male.[8] CX-1 is a moderately well-differentiated adenocarcinoma consistent with gastrointestinal origin, surgically removed from the primary tumor of a 44-year-old female.[9] GI-117 is a poorly differentiated carcinoma established from a tumor of a 62-year-old female.[10] PC3 is a grade IV adenocarcinoma from a 62-year-old Caucasian.[11] GI-112 is a moderately to poorly differentiated ade-

* To whom correspondence should be addressed. e-mail: terasaki@mail.pharm.tohoku.ac.jp

Table 1. Primer Sets for RT-PCR Analysis

Gene	Accession No Product size	Sense primer sequence Anti-sense primer sequence
ABCA5	NM_01872 362 bp	GGACTGGATAGAAAACCTAGGAAGTAGACC GAAAGAAGTCCCAGTAAGCAGACCGAAC
ABCA6	NM_080284 533 bp	CCAGGAGCACTACAGAGAATTTCCAG AGGTGGCCCAAAACTGAACTGCAG
ABCA8	NM_007168 533 bp	CTGGCTGGTTTTAACATCGAGTTGCC TAGGAATACCAGAAATGGCTGTACATCC
ABCA9	NM_080283 501 bp	GGATACCAATGGCAGCCTCTTTCAC CTCCTAAGTAATCCATGGAATCAGGAG
ABCA10	NM_080282 565 bp	GACTTTATGAGAAACTTGGACAGTCTGG CCAAGAACTTGAGGCTGTTGTCATGC
ABCB1	NM_000927 353 bp	GACAGAAAGCTTAGTACCAAAGAGGCTC GATCGGAAAACCATGTATCGGAGCCG
ABCC1	NM_004996 496 bp	AGCTAGACCATGAATGTGCAGAAGGC GTGGCTGCTGCTTTGAATATGTTTTGG
ABCA2	NM_001606 562 bp	GCAGCCAGAGTGTGAAGGACGTGG AACCTCCTCTCCAGGCCCTGGCA
β-Actin	NM_001101 348 bp	TTTGAGACCTTCAACACCCC TAGCTCTTCTCCAGGGAGG

nocarcinoma established from a 54-year-old female.[10] GI-102 is an undifferentiated carcinoma isolated from a primary ovarian carcinoma.[10] GI-103 is a poorly differentiated carcinoma propagated from ascetic fluid derived from pancreatic adenocarcinoma.[10]

PCR Analysis PCR was conducted with specific primer sets (Table 1) through 1 cycle of 94 °C for 30 s, 2 cycles of 94 °C for 30 s and 72 °C for 2 min, 3 cycles of 94 °C for 30 s and 70 °C for 2 min, and 25 cycles of 94 °C for 30 s and 68 °C for 2 min. The RT-PCR products were separated by electrophoresis on agarose gel. All PCR products from human brain were subcloned and sequenced using a DNA sequencer (CEQ2000XL; Beckman Coulter, Fullerton, CA, U.S.A.).

RESULTS AND DISCUSSION

Tissue Distributions of Human ABCA5-Like Transporters Expression of ABCA5-like transporter mRNAs in human tissues was examined by RT-PCR (Fig. 1). The mRNA expressions of these transporters exhibited similar distribution patterns, which may be related to the fact that the genomic loci of these transporters are located close together.[3] Strong expression of all the transporter mRNAs was detected in the brain, and the nucleotide sequence of each product was identical with that of the corresponding gene. ABCA5 and ABCA8 showed similar mRNA expression profiles in spleen, testis and ovary. Strong ABCA5 mRNA expression was also detected in liver and pancreas. In addition, strong ABCA6 mRNA expression was detected in lung and liver, and ABCA8 mRNA was detected in lung.

Expression of ABCA5-Like Transporters in Human Tumors Figure 2 shows the expression of human ABCA5-like transporter mRNAs in various human tumors as determined by means of RT-PCR analysis. ABCA6, ABCA7 and ABCA8 mRNAs were not detected in any of the examined tumors, but weak expression of ABCA10 was detected in all of them. ABCA5 mRNA was strongly expressed in GI-112

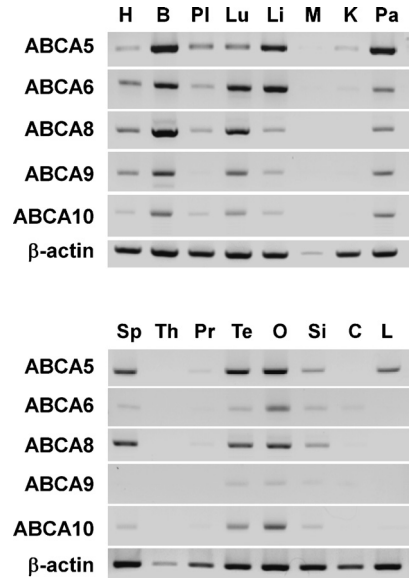

Fig. 1. Tissue Distributions of mRNA Expression of Human ABCA5-Like Transporters

RT-PCR analysis was performed with the specific primer sets shown in Table 1. H, heart; B, brain; Pl, placenta; Lu, lung; Li, liver; M, skeletal muscle; K, kidney; Pa, pancreas; Sp, spleen; Th, thymus; Pr, prostate; Te, testis; O, ovary; Si, small intestine; C, colon; L, peripheral blood leukocytes.

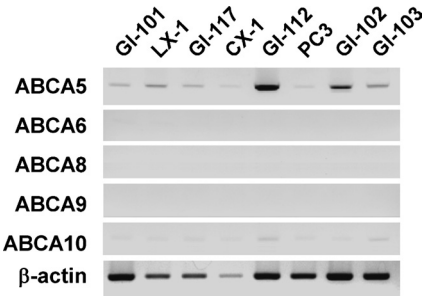

Fig. 2. Tumor Distributions of mRNA Expression of Human ABCA5-Like Transporters

RT-PCR analysis was performed with the specific primer sets shown in Table 1. GI-101, breast carcinoma; LX-1, lung carcinoma; GI-117, lung carcinoma; CX-1, colon adenocarcinoma; GI-112, colon adenocarcinoma; PC3, prostatic adenocarcinoma; GI-102, ovarian carcinoma; GI-103, pancreatic adenocarcinoma.

and GI-102, which are moderately to poorly differentiated colon adenocarcinoma and undifferentiated ovarian carcinoma, respectively. Though normal human ovary also expresses ABCA5 mRNA, as shown in Fig. 1, ABCA5 mRNA was not detected in normal colon, suggesting that its expression was induced in colon tumor. In CX-1, a well differentiated colon adenocarcinoma, ABCA5 mRNA was only faintly expressed. However, the low mRNA content in CX-1 may be partly artifactual, because the β-actin mRNA level was lower than in other samples.

Expression of Human ABCA5 and Drug-Resistance Genes in Human Colon Carcinomas Some ABC transporters play a role in drug resistance; their expression is induced in cancer cells, and they mediate the efflux transport of drugs from the cells. The expression of human ABCA5 in normal colon and colon carcinomas was compared with those of ABC transporters known to be involved in drug resistance, such as ABCB1 (MDR1), ABCC1 (MRP1) and ABCA2.[1,12] The results shown in Fig. 3 indicate that there

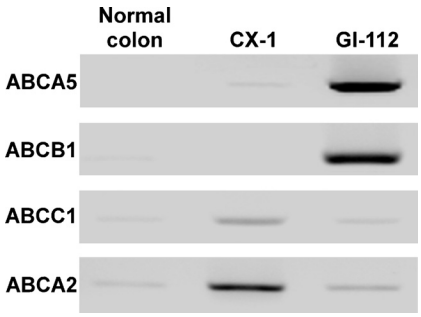

Fig. 3. mRNA Expression of Human ABCA5 and Drug Resistance-Associated ABC Transporters in Normal Colon and Colon Adenocarcinomas

RT-PCR analysis was performed with the specific primer sets shown in Table 1. CX-1, well differentiated colon adenocarcinoma; GI-112, poorly differentiated colon adenocarcinoma.

are different expression profiles of ABC transporters in CX-1 and GI-112. In GI-112, ABCA5 and ABCB1 mRNAs were predominantly expressed, while ABCC1 and ABCA2 mRNAs were predominantly expressed in CX-1. Since GI-112 is a moderately to poorly differentiated adenocarcinoma and CX-1 is a well-differentiated adenocarcinoma from a primary tumor, the difference in expression profile may be related to the differentiation stages of the tumors. Furthermore, the results suggest that induction of ABCA5 in colon carcinoma is linked with ABCB1 induction.

This is the first demonstration that expression of ABCA5 is induced in tumors, and further that it may be linked with ABCB1 induction, and may be correlated with the tumor differentiation state. Since progression of malignancy is associated with dedifferentiation, poorly differentiated carcinoma shows a higher grade of malignancy than well-differentiated carcinoma. The introduction of a c-H-ras-1 oncogene into a poorly differentiated human colon carcinoma cells has been reported to result in significant reduction of ABCB1 mRNA expression and P-glycoprotein-mediated drug resistance in association with acquisition of a more differentiated phenotype.[13] This suggested that the change in the expression profile of ABC transporters might be related to the properties of the tumors in each differentiation stage. One possible function of ABCA5 in tumors would be as a drug efflux transporter, which might result in multi-drug resistance, in association with ABCB1. Another possibility is that ABCA5 transport function is related to tumor differentiation. A study in ABCA5 knockout mice showed decreased plasma levels of thyroid hormones,[5] suggesting a relationship between ABCA5 and thyroid hormones. It is conceivable that ABCA5 transports thyroid hormones directly, or transports compounds that regulate thyroid hormone homeostasis. Thyroxine (T4) enhances the development of colon tumors,[14] and levothyroxine (L-T4) upregulates expression of ABCB1 in human colon carcinoma cells, Caco-2.[15] These findings are consistent with the idea that ABCA5 function is related to the differentiation state of colon tumors.

In conclusion, we have measured the mRNA expression profile of ABCA5-like transporters in human tissues and tumors. Our results indicate that ABCA5 mRNA expression is induced in human colon tumors, and this induction is linked with induction of ABCB1 mRNA expression. It is now important to clarify the function of ABCA5 in order to understand its putative role in tumor differentiation and drug resistance.

Acknowledgements This study was supported in part by a Grant-in-Aid for a Scientific Research on Priority Areas 17081002 from The Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, and a 21st Century Center of Excellence (COE) Program grant from the Japan Society for the Promotion of Science. It was also supported in part by the Industrial Technology Research Grant Program from the New Energy and the Industrial Technology Development Organization (NEDO) of Japan.

REFERENCES

1) Sarkadi B., Homolya L., Szakacs G., Varadi A., *Physiol. Rev.*, **86**, 1179—1236 (2006).
2) Takahashi K., Kimura Y., Nagata K., Yamamoto A., Matsuo M., Ueda K., *Med. Mol. Morphol.*, **38**, 2—12 (2005).
3) Annilo T., Chen Z. Q., Shulenin S., Dean M., *Mamm. Genome*, **14**, 7—20 (2003).
4) Tsuruoka S., Ishibashi K., Yamamoto H., Wakaumi M., Suzuki M., Schwartz G. J., Imai M., *Biochem. Biophys. Res. Commun.*, **298**, 41—45 (2002).
5) Kubo Y., Sekiya S., Ohigashi M., Takenaka C., Tamura K., Nada S., Nishi T., Yamamoto A.,Yamaguchi A., *Mol. Cell. Biol.*, **25**, 4138—4149 (2005).
6) Petry F., Ritz V., Meineke C., Middel P., Kietzmann T., Schmitz-Salue C., Hirsch-Ernst K. I., *Biochem. J.*, **393**, 79—87 (2006).
7) Hurst J., Maniar N., Tombarkiewicz J., Lucas F., Roberson C., Steplewski Z., James W., Perras J., *Br. J. Cancer*, **68**, 274—276 (1993).
8) Anderson W. K., *Cancer Res.*, **42**, 2168—2170 (1982).
9) Schmid F. A., Sirotnak F. M., Otter G. M., DeGraw J. I., *Cancer Treat. Rep.*, **69**, 551—553 (1985).
10) Ni X., Gu S., Dai J., Cheng H., Guo L., Li L., Ji C., Xie Y., Ying K., Mao Y., *J. Hum. Genet.*, **48**, 96—100 (2003).
11) Kaighn M. E., Narayan K. S., Ohnuki Y., Lechner J. F., Jones L. W., *Invest. Urol.*, **17**, 16—23 (1979).
12) Boonstra R., Timmer-Bosscha H., van Echten-Arends J., van der Kolk D. M., van den Berg A., de Jong B., Tew K. D., Poppema S., de Vries E. G., *Br. J. Cancer*, **90**, 2411—2417 (2004).
13) Kramer R., Weber T. K., Arceci R., Morse B., Simpson H., Steele G. D., Jr., Summerhayes I. C., *Int. J. Cancer*, **54**, 275—281 (1993).
14) Iishi H., Tatsuta M., Baba M., Okuda S., Taniguchi H., *Int. J. Cancer*, **50**, 974—976 (1992).
15) Mitin T., Von Moltke L. L., Court M. H., Greenblatt D. J., *Drug Metab. Dispos.*, **32**, 779—782 (2004).