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Functional analysis of ABCA8, a new drug transporter

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Abstract

We examined the transport capacity in *Xenopus laevis* oocytes of human EST KIAA0822/ABCA8, a member of the ABC superfamily. Substrates of ABCC2/MRP-2 such as [^{14}C]estradiol-β-glucuronide, taurocholate, and LTC4, and of organic anion transporter (OAT), such as *para*-aminohippuric acid, ochratoxin-A, were significantly accumulated while tetraethylammonium and doxorubicin were not. The transport of [^{14}C]estradiol-β-glucuronide was ATP-dependent and K_m and V_{max} values of 30.4 μM and 66.9 pmol/h/egg, respectively, were estimated. The transport of [^{14}C]estradiol-β-glucuronide was inhibited by substrates/inhibitors of ABCC2/MRP-2, but not by those of the organic cation transporter and multidrug resistance protein (MDR)-1. KIAA0822/ABCA8 possesses two ATP-binding sites and fourteen transmembrane domains. Northern blot analysis revealed expression in most organs, especially in heart, skeletal muscle, and liver. Thus, ABCA8 is a new member of the xenobiotic transporter ABC-subfamily.

Keywords: ABC-family; Estradiol-β-glucuronide; Organic anion; ABCA8

The ABC superfamily comprises more than 400 proteins and is widely distributed from bacteria to human [1]. Some members of the family, such as ABCB1 (previously called MDR-1) and ABCC2 (previously called MRP-2), are efflux pumps of hydrophobic drugs [2]. ABCC2/MRP-2 secretes xenobiotics into the bile duct and mutations in this gene are responsible for the Dubin–Johnson syndrome [3].

We found a member of the human ABC-superfamily (KIAA0822/ABCA8) in the EST-database and determined its function by expressing its cRNA into it Xenopus laevis oocytes. We evaluated the transport properties for several drugs, which are substrates of other ABC-drug transporters. Because some substrates of ABCC2 overlap with the organic anion transporter (OAT), we also evaluated transport capacity for some OAT substrates. We also determined the human tissue distribution of the gene by Northern blot.

Methods

Database screening for new member of ABC superfamily. We screened the GenBank database by the BLAST program using several known ABC transporters including the P-glycoprotein as the query. We found a *Homo sapiens* complete cDNA (AB020629, the mRNA of which is 5677 bp), which is now called ABCA8 by the HUGO (Human Gene Organization) Nomenclature Committee. This cDNA was obtained in pBluescript SK(−) from Kazusa DNA Research Institute (Kisarazu, Japan). The Kyte-Doolittle method predicted that the expressed cDNA possesses 14 transmembrane domains.

Preparation of Xenopus laevis oocytes and micro-injection of RNA. Mature *Xenopus laevis* females were purchased from Hamamatsu Animal (Shizuoka, Japan) and kept under standard conditions. Stage V oocytes were selected and incubated in modified Barth's solution (MBS) containing 88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 15 mM Tris–Cl, 0.3 mM $CaNO_3$, 0.82 mM $MgSO_4$, and 0.41 mM $CaCl_2$ with 10 μg/ml penicillin and 10 μg/ml streptomycin. Mature healthy oocytes were injected with 50 nl transcribed 5′-capped-cRNA in vitro or water using an automatic injector (IM 200J, Narishige, Japan) as described previously [4]. Injected oocytes were cultured at 17 °C for 3 days in MBS with daily changing of the medium.

Evaluation of drug transport capacity. The cultured oocytes ($n = 10$–20) were transferred to small chamber with 2–3 ml medium and radioisotope. After isotope uptake, each oocyte was washed three times with MBS and dissolved with 10% SDS for 30 min at room

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temperature. We used [^{14}C]estradiol-β-glucuronide [5,6] and leucotriene C4 (LTC4) [7] as probes for ABCC2 (formerly called MRP-2). We used [^{3}H]TEA (tetraethylammonium) and [^{3}H]digoxin as probes for OCT (organic cation transporter) and MDR (multidrug resistance protein), respectively. We also used estrone sulfate, PAH, ochratoxin A, and LTC4 as probes for organic anion transporters (OATs). The oocyte-related radioactivity was measured by liquid-scintillation.

Determination of tissue distribution by Northern blot. A transfer filter with human total RNA from various organs was purchased from Clontech (Human 12-Lane MTN Blot; Palo Alto, CA). Each lane contained 2 μg polyadenylated RNA from various human tissues. The filter was hybridized for 3 h at 68 °C in hybridization solution (ExpressHyb, Clontech) containing a probe (full length of the cDNA) labeled with [α-^{32}P]dCTP by random priming. The filter was washed in 0.1× SSC and 0.5% SDS at 55 °C for 30 min, and then exposed to X-ray film at −70°C between two intensifying screens.

Results

Screening cDNA libraries

The complete *Homo sapiens* cDNA corresponding to the KIAA0822 protein (AB020629, 5677 bp mRNA) fulfilled the criteria of our screening. The cDNA was originally cloned as one of the full-length cDNAs with unknown function in human brain by Ohara and coworkers [8] from Kazusa DNA Research Institute (Kisarazu, Chiba, Japan) and registered in GenBank (with Accession No. AB020629). The gene is now called ABCA8 by the HUGO (Human Gene Organization) Nomenclature Committee. However, the function of this protein was not determined [http://www.gene.ucl.ac.uk/nomenclature/genefamily/abc.html]. Hydropathy analysis indicated that the corresponding protein sequence possessed 14 predicted transmembrane domains. It also contained two putative ATP-binding cassettes. However, the ABC motif –LSGGQ– motif, which is commonly observed in other ABC families [9], was not detected. We obtained the cDNA in pBluescript SK(−) from the Kazusa DNA Research Institute, expressed it, and characterized its function in the following experiments.

Transport properties

In oocytes injected with RNA, the uptake of [^{14}C]estradiol-β-glucuronide linearly increased with time until about 60 min where it reached 138.0 ± 5.5 fmol/h/egg while it was almost negligible (2.7 ± 0.4 fmol/h/egg) in the water-injected group. The uptake followed Michaelis–Menten kinetics (Fig. 1). Estimated K_m and V_{max} were 30.4 μM and 66.9 pmol/h/egg, respectively. We evaluated the ATP-dependence of the uptake of [^{14}C]estradiol-β-glucuronide in the medium. The uptake was increased by the addition of ATP up to 5 mM (294 ± 8, 445 ± 15, 521 ± 19, and 529 ± 32 fmol/h/egg with 1, 2, 5, and 10 mM ATP, respectively). Furthermore, the transport was completely abolished by addition of 10 mM potassium cyanide to the medium.

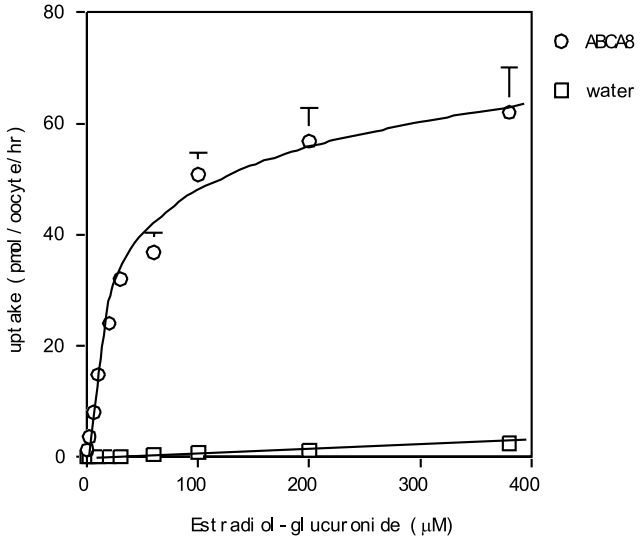

Fig. 1. Kinetics of [^{14}C]estradiol-β-glucuronide uptake in *Xenopus* oocytes.

To determine the substrate selectivity in more detail, we examined the inhibitory effect of various compounds on the uptake of [^{14}C]estradiol-β-glucuronide (Fig. 2). The cis-inhibitory effect was observed by addition of MS-209 and MK-571, potent inhibitors of ABCC2/MRP-2 [10,11]. The uptake was reduced to about 30% by addition of 10 μM of these agents. Ochratoxin A (50 μM), a substrate of ABCC2/MRP-2 [12], also inhibited transport. Verapamil (1 mM) and digoxin

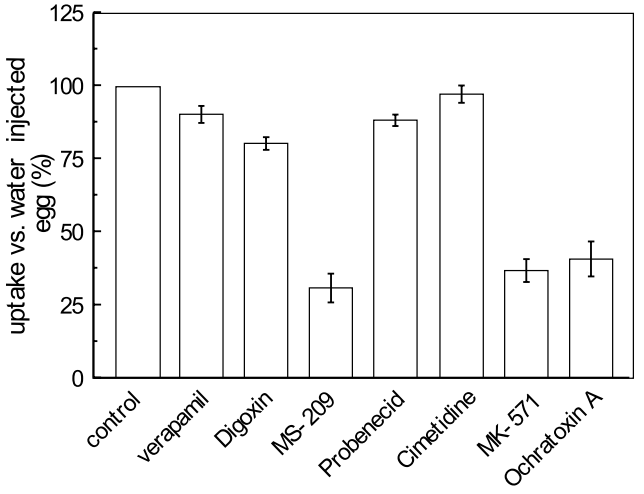

Fig. 2. Inhibitory effect of various xenobiotics on [^{14}C]estradiol-β-glucuronide uptake in RNA-injected eggs. Concentration of the [^{14}C]estradiol-β-glucuronide in the bathing solution was 37 nM. Verapamil (1 mM), digoxin (0.25 mM), MS-209 (10 μM), probenecid (1 mM), cimetidine (100 μM), MK-571 (100 μM), and ochratoxin A (50 μM) were added with the isotope.

Table 1
K_m and V_{max} of various substrates

Substrates	K_m (μM)	V_{max}
LTC4	0.1	61 fmol/egg/h
Taurocholate	10.3	11.0 fmol/egg/h
PAH	5.0	79 fmol/egg/h
Estrone sulfate	0.5	1.7 pmol/egg/h
Ochratoxin A	0.4	2.1 pmol/egg/h

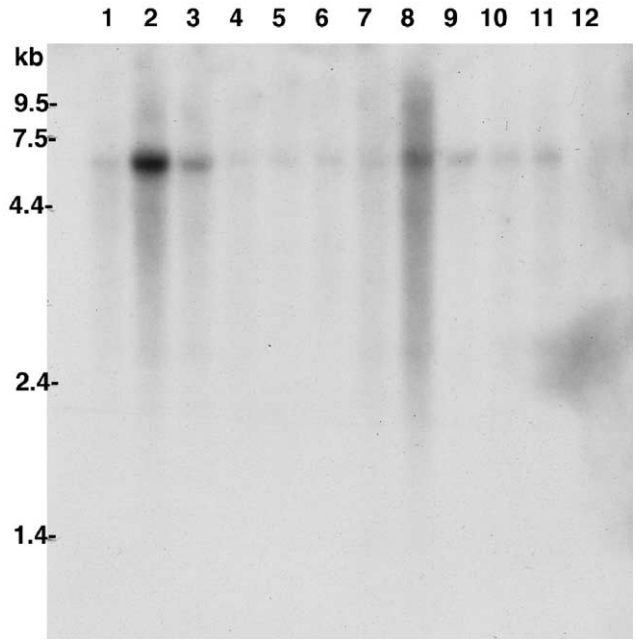

Fig. 3. Northern blot analysis of human tissues. A Human 12-Lane MTN Blot (Clontech) was hybridized with a probe of full length of human ABCA8 cDNA. Each lane contained 2 μg poly(A) RNA of various human tissues. The positions of the size-markers are indicated on the left. The numbers represent the tissues: 1, brain; 2, heart; 3, skeletal muscle; 4, colon; 5, thymus; 6, spleen; 7, kidney; 8, liver; 9, small intestine; 10, placenta; 11, lung; 12, peripheral blood leukocytes.

(0.25 mM), substrates of ABCB1/MDR-1 [13], also showed small but significant inhibition of the uptake. Probenecid, a substrate of OAT, showed small inhibition similar to digoxin, while cimetidine, a substrate of OCT, did not affect the uptake.

To further evaluate the substrate specificity of the transporter, we also determined the uptake of other radioactive chemicals (Table 1). LTC4 and taurocholate, which are, respectively, substrates of ABCC2/MRP-2 [5] and of oatp,a member of the multispecific organic anion transporter family [14], were transported. The uptake showed saturable Michaelis–Menten kinetics (data not shown). K_m values of 0.1 μM for LTC4 and 10.3 μM for taurocholate were observed. The expressed ABCA8 transported the OAT substrates, PAH and estrone sulfate, although the transport capacity was not high (about 2–3-fold higher than control egg). K_m values were 5 μM for PAH and 0.5 μM for estrone sulfate. Transport of ochratoxin A, another substrate of OAT, was about 12-fold higher than control). However, 10 nM TEA, a substrate of OCT, was not transported. Surprisingly, the uptake of 100 nM digoxin was smaller than that in water-injected oocytes (80 ± 14% of uptake compared with water-injected egg).

Tissue distribution of ABCA8 mRNA

Fig. 3 shows the results of a Northern blot of 2 μg of various tissue poly(A) RNAs. We can compare the tissue distribution of ABCA8 mRNA without an internal standard The expression was detected in most organs, especially in heart, skeletal muscle, and liver. Although this gene was originally obtained from a cDNA library of brain, the signal in the brain was lower than from those organs.

Discussion

By screening an EST-database, we found KIAA0822 (now called ABCA8) as a possible new member of the ABC-transporter superfamily. This ABCA8 protein was shown to transport several drugs with a specificity substrate close to that of ABCC2/MRP-2 and of oatp, which does not belong to the ABC superfamily. The transport maximum is ATP-dependent. The K_m of ABCA8 for the transport of [^{14}C]estradiol-β-glucuronide was surprisingly low and similar to that of MRP-2/ABCC2 in Caco-2 cells [6], transfected insect cells [10], and HeLa-T14 cells [5]; however, the amino-acid sequences of KIAA0822/ABCA8 and ABCC2/MRP-2 were only 15% identical. It was reported that [^{14}C]estradiol-β-glucuronide is a substrate for the multispecific organic anion transporters, oatp (1, 2, 3). Because the K_m value of [^{14}C]estradiol-β-glucuronide for ABCA8 was similar to that for ABCC2 and about 10-fold lower than that for oatp1, 2, and 3, [15], the affinity to [^{14}C]estradiol-β-glucuronide of this clone is much closer to that of ABCC2/MRP-2 than to that of oatp. Despite the similar K_m values for [^{14}C]estradiol-β-glucuronide of ABCA8 and ABCC2/MRP2, the K_m for LTC4 of ABCA8 was lower than those of ABCC2/MRP2. This difference distinguished ABCA8 from ABCC2/MRP2.

We also found that ABCA8 transports substrates of the organic anion transporter (OAT). In particular, the transport of ochratoxin A was compatible with that of OAT3. The K_m and V_{max} values of ABCA8 for PAH and estrone sulfate, other substrates for OAT3, were lower than for OAT3.

KIAA0822/ABCA8 was predicted to have 14 transmembrane domains. The two putative ATP-binding sites are consistent with this protein being a member of the ABC-family. Whereas motifs-A (Walker A) and -B (Walker B) were found in its protein sequence, motif-C

(–LSGGQ) was not observed. This is different from previously known ABC-proteins. In contrast, it was recently reported that a full clone of ABCA6 was 60% identical to ABCA8, even though the function of ABCA6 was not determined [16]. The motif-C was not found in the protein sequence of ABCA6, either.Homology analysis of other ABC-A-families shows 29% amino acid identity to ABCA4, the disruption of which causes Stargardt muscular dystrophy [17] and 30% identity to ABCA1, the mutation of which causes Tangier's disease [18].

ABCA1 works as an anion pump when expressed in oocytes [19] as well as a transporter of lipid [20,21]. The anion-drug specificity of KIAA0822/ABCA8 is related to that of ABCA1. Although most genes belonging to the ABC-A subgroup can transport lipids, such transport has not been examined yet in ABCA8; further studies are needed.

Although the substrate-specificity of ABCA8 is close to that of ABCC2/MRP-2, as well as to that of multispecific organic anion transporters, the cellular accumulation of radiolabeled digoxin was slightly but significantly lower than that in water-injected one. This is different from that of ABCC2, which transports digoxin at high rates [10]. Noe et al. [15] reported that oatp2 (TC# 2.A.60.1.5), a member of the organic anion transporters, rapidly transports digoxin, as well as some organic anions.

The expression of mRNA by Northern blot was widely distributed in various organs. It is especially well expressed in heart, skeletal muscle, and liver, which is similar to other ABC-A families. Because the physiological and patho-physiological roles of this gene product have not been examined, further study especially in pathological conditions of the heart and liver is indicated.

In summary, we screened an EST-database and found a new member of the ABC superfamily, which is now named ABCA8. We also found that it functions as an ATP-dependent drug transporter. The substrate specificity for the transport of [^{14}C]estradiol-β-glucuronide was close to that of ABCC2/MRP-2 within the ABC superfamily and also partly similar to other proteins not belonging to the ABC family.

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