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The ATP-binding cassette transporter ABCA2 as a mediator of intracellular trafficking

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Abstract

ATP-binding cassette (ABC) transporters are a family of proteins that translocate molecules across cellular membranes. Substrates can include lipids, cholesterol and drugs. Mutations in ABC transporter genes can cause human pathologies and drug resistance phenotypes in cancer cells. ABCA2, the second member the A sub-family to be identified, was found at high levels in ovarian carcinoma cells resistant to the anti-cancer agent, estramustine (EM). In vitro models with elevated levels of ABCA2 are resistant to a variety of compounds, including estradiol, mitoxantrone and a free radical initiator, 2,2′-azobis-(2-amidinopropane) (AAPH). ABCA2 is most abundant in the central nervous system (CNS), ovary and macrophages. Enhanced expression of ABCA2 and related proteins, including ABCA1, ABCA4 and ABCA7, is found in human macrophages upon bolus cholesterol treatment. ABCA2 also plays a role in the trafficking of low-density lipoprotein (LDL)-derived free cholesterol and is coordinately expressed with genes involved in cholesterol homeostasis. Additionally, ABCA2 expression has been linked with gene cluster patterns consistent with pathologies including Alzheimer's disease (AD). A single-nucleotide polymorphism (SNP) in exon 14 of the ABCA2 gene was shown to be linked to early onset AD in humans, supporting the observation that ABCA2 expression influences levels of β-amyloid peptide (Aβ), the primary component of senile plaques. ABCA2 may play a role in cholesterol transport and affect a cellular phenotype conducive to the pathogenesis of a variety of human diseases including AD, atherosclerosis and cancer.

Keywords

ABC transporters; Drug resistance; Estramustine; Cholesterol; Alzheimer's disease

1. Introduction

ATP-binding cassette (ABC) transporters are multi-domain membrane proteins that use energy from ATP hydrolysis to pump substrates directionally across cellular membranes [1]. The human ABC superfamily of proteins consists of at least seven sub-families: A (ABC1);

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B (MDR/TAP); C (CFTR/MRP); D (X-linked adrenoleukodystrophy, ALD); E (OABP); F (GCN20); G (WHITE). A wide diversity of tissue expression has been reported. Expression patterns are also influenced by genetic polymorphisms, some of which have been associated with human disease pathologies. Reports have shown at least 605 single-nucleotide polymorphisms (SNPs) among 13 ABC transporter genes [2]. ABC transporters have a generic structure composed of two transmembrane domains and two ABCs [3]. The ABC consensus sequence is the hallmark of the ABC protein superfamily and is comprised of highly conserved Walker A and B motifs separated by 90–120 amino acids, within which there is a characteristic “signature” motif [4]. Inhibition of hydrolysis of one ABC domain can abrogate the activity of the other and thereby function in an alternating fashion while recognizing diverse nucleotides as hydrolytic substrates. Topology analysis has shown that the number of trans-membrane helices can range from 6 to 11 [5], and these function in substrate recognition, binding, and channeling. ATP hydrolysis in the ABC domain provides the necessary energy for substrate transport. Interactions between the ABC and membrane domains are integral in executing energy dependent transport. Within the A sub-family (ABCA) there are seven members that function in tissue-specific fashions. Table 1 provides a summary of ABCA sub-family function and association with disease; however broader reviews of transporter structure/function are available in the literature [6–10].

2. ABCA2

ABCA2 (270 kDa) is the largest of 49 identified members of the ABC transporter gene family (Fig. 1) [11]. This transporter was originally described along with ABCA1 in embryonic mouse brain [12,13]. Subsequent characterization of the full-length human *ABCA2* cDNA and its detailed expression pattern showed that ABCA2 is most highly expressed in both fetal and adult brain, spinal cord, ovary and leukocytes [14]. Recently, ABCA2 protein was shown to localize in areas of the brain associated with adult neurogenesis and Alzheimer’s disease (AD) pathology (subventricular zone lining of the lateral ventricles and the dentate gyrus of the hippocampus), as well as in GABAergic and glutamatergic neurons [15]. Expression of ABCA2 has been detected in other tissues, including in descending order: lung, kidney, heart, liver, skeletal muscle, pancreas, testis, spleen and fetal liver. Cellular immunolocalization of ABCA2 revealed a distinct, punctate staining that vesicle-specific antibodies revealed to be a co-localization of ABCA2 with late endolysomes and trans-golgi organelles.

The ABCA2 gene is located at chromosome 9q34 within a genomic region of 21 kb [16]. The gene contains 48 exons with an open reading frame of 2436 amino acids [14,17]. The minimal promoter region has been mapped to 321 bp upstream of the translation start site [18]. Alternative splicing of the first exon to the second in ABCA2 results in two variants, 1A and 1B [19]. The first exon of 1B contains the coding sequence for 52 amino acids and is located 699 bp upstream of 1A, which contains coding sequence for 22 amino acids. Both splice variants co-localize with lysosome-associated membrane proteins-1 and −2 (LAMP-1 and −2) and share similar expression profiles [19]. The novel N-terminus of ABCA2 splice variants, while functionally redundant, may provide subtle gene regulatory differences to allow tissue-specific or temporal-specific protein expression during differentiation and/or development.

ABCA2 shares homology with other A sub-family proteins, including ABCA1 (50%) ABCA7 (44%), ABCA3 (43%), ABCA4 (40%) and ABCA6 (32%) [20]. Promoter elements identified in ABCA1 include an E box, AP-1, liver X receptor (LXR) element and SP1 motifs [21]. The proximal promoter of ABCA2 contains two GC-boxes and overlapping sites for the early growth response-1 (EGR-1) and Sp1 transcription factors [18]. While there are identical regions in ABCA1 and ABCA2 (e.g. two cytoplasmic ABCs, Walker domains, a conserved N-terminal sequence (LLLWKN) and a VFVNFA motif within the C-terminal domain [22]), their functional overlap may not be significant. The VFVNFA motif in ABCA1 is critical for apolipoprotein A-I binding and high density lipoproteins (HDL) cholesterol efflux at the plasma membrane [22]. Nevertheless, the ABCA2 sequence contains a lipocalin signature motif, implying a function in the transport of lipids, steroids and structurally similar molecules.

3. Cholesterol homeostasis and metabolism

Several of the ABC sub-family A members play a role in cholesterol homeostasis (Table 1). Specifically, ABCA1 and ABCA7, transporters that not only share the greatest homology with ABCA2, but are also sterol responsive genes functioning in cholesterol metabolism [20]. Cholesterol homeostasis is maintained by a feedback mechanism of de novo synthesis using acetyl CoA and by uptake of low-density lipoprotein (LDL)-cholesterol by receptor-mediated endocytosis through the LDL receptor (LDLR) [23]. Functional studies using exogenous, labeled LDL cholesterol provided evidence for ABCA2 function in sterol trafficking [24]. Forced ABCA2 overexpression was used as a model system in Chinese hamster ovary cells (CHOA2) to test the effect on transport of LDL-cholesterol. CHOA2 cells displayed a more intense staining of unesterified cholesterol using the fluorescent marker, filipin, in cytoplasmic and perinuclear vesicles, compared to the parental cell line (CHO). These vesicles were also positive for an endosome/lysosome acidic vesicle marker (Lysosensor green 189), with less intense filipin staining at the plasma membrane [24]. In addition to the sequestration of LDL-derived cholesterol into these vesicles, CHOA2 cells have also been shown to have elevated expression of the LDLR, sterol-response element binding protein-2 (SREBP2) and 3-hydroxy-3-methylglutaryl CoA synthase (HMGCoA S) and demonstrate reduced trafficking of LDL-derived cholesterol to the endoplasmic reticulum (ER) for esterification [24]. Since these cellular responses also occur when cells are grown under sterol-depleted conditions, these data suggest that ABCA2 up-regulation mimics sterol deprivation. LDLR (along with HMGCoA S and SREBP2) is regulated by the SREBP2 transcription factor [25]. The transcription of LDLR is reduced in CHOA2 cells with a mutant SREBP2 binding site within the LDLR promoter [18]. Taken together, these results show that ABCA2 over-expression causes a phenotype similar to cholesterol-depleted cells that sequester unesterified cholesterol into endolysosomal compartments.

Significant insight into the importance of cholesterol homeostasis has been gleaned from pre-clinical studies of ABCA1. For example, ABCA1 deficient mice have a ~70% decrease in serum cholesterol, phospholipids and lack HDL [26], while transgenic overexpressing mice show an increase in cholesterol efflux [27]. Cholesterol lowering agents, such as statins, impact ABCA1 expression, resulting in a reduction in intracellular cholesterol [28]. The significance of ABCA1 and dysregulation of cholesterol metabolism is noted in the

mouse model for diabetes with associating cardiovascular disease. ABCA1 expression is decreased in diabetic mice with cardiovascular disease [29]. Although the generation of mouse models for other ABC A sub-family transporters began only recently [30–32], there will undoubtedly be increasing numbers of genetic models that should help to elucidate in vivo functions and genetic links with human disease.

3.1. ABCA2 and disease

ABCA2 expression is elevated in Niemann-Pick type C1 (NPC1) fibroblasts and in Familial Hypercholesterolemia (FHC) fibroblasts [24], as well as several cancer cell lines including an estramustine (EM)-resistant prostate cancer cell line, EM15. Although ABCA2 is expressed predominantly in normal and malignant central nervous system (CNS) tissues and cell lines, it is also abundant in several other cancer cell lines derived from non-CNS tissues. The expression of ABCA2 in oligodendrocytes is purported to be required for the generation of copious layers of phospholipid- and cholesterol-rich myelin sheath of white matter in the brain. The cell lines that express the highest levels are not derived from the CNS, suggesting a putative role for the deregulation of ABCA2 in tumorigenesis and/or cancer progression. However, the mounting evidence for a putative role of ABCA2 in the development of AD may lead to its use as a target for future therapeutic strategies.

AD is the most common neurological pathology associated with dementia and manifests itself by the accumulation of extracellular senile plaques in neurofibrillary tangles of the brain and cerebral blood vessels. These plaques are composed of both fibrillar and non-fibrillar forms of β-amyloid peptide (Aβ) and result in the loss of neuronal function in limbic and association cortices of the brain [33]. The Aβ peptide is derived from sequential cleavage reactions of amyloid precursor protein (APP) by a β-secretase, followed by cleavage by a γ-secretase within the transmembrane domain of APP. Approximately 40% of early-onset AD cases (in individuals younger than 60 years) are linked to mutation in the β-amyloid precursor protein (APP), presenilin1 (PSEN1), or presenilin2 (PSEN2) [34]. Late-onset AD occurs in patients older than 60 and accounts for nearly 95% of all AD cases. Several genes have shown a slight, but inconsistent association with this disease [35], but the most common of these is the ε4 isoform of APOE [33,36]. There is mounting epidemiological and genetic research that has drawn a close link between AD pathology and cholesterol [37–40]. Mid-life individuals with elevated serum cholesterol have an increased risk of developing AD [41] and treatment with statins, cholesterol lowering agents, is associated with a decreased risk of AD development [42] and a decrease in Aβ levels [43].

As a cholesterol-responsive gene expressed predominantly in the brain, ABCA2 became an excellent candidate for an association with AD when it was recently shown to impact the production of Aβ. Not only did ABCA2 co-localize with Aβ and APP [44], its overexpression also caused an up-regulation of a number of genes associated with resistance to oxidative stress. Using amplified differential gene expression (ADGE) microarray, several clusters of genes were shown to be differentially regulated upon ABCA2 over-expression in HEK293 cells, including 22 genes related to transport, membrane homeostasis, cell metabolism and substrate binding. Six of the genes from this study, APP, LDLR-related protein, calcineurin, seladin-1, vimentin and Slc23a1, are commonly associated with AD and

response to oxidative stress. Overexpression was also shown to confer a slight resistance to oxidative stress rendered by the free radical initiator, 2,2′-azobis-(2-amidinopropane) (AAPH). ABCA2 levels were shown to be elevated in the temporal and frontal regions of the brain, areas frequently associated with AD pathology. Recently, a synonymous SNP in exon 14 of ABCA2 (rs908832) was determined to have a significant linkage with early-onset AD [45]. Taken together, these studies reflect the importance of determining a mechanistic role for ABCA2 in AD pathogenesis and/or progression. However, because cholesterol transport is critical to a number of important phenotypes, the ABCA2 protein may impact other diseases such as cancer.

Hormone therapy of cancer patients has been shown to influence cholesterol metabolism [46,47]. Cholesterol also has critical functions in cell signaling, survival and differentiation [48] and may provide a novel target for cancer therapeutic agents [49] since cholesterol levels may be a limiting factor in membrane maintenance in rapidly dividing cancer cells. Although many cellular factors influence tumor cell proliferation, the role of the availability of growth-promoting metabolites is an important area of investigation. The potential role of ABCA2 in regulating availability of cholesterol as a critical metabolite for tumor cell proliferation may validate it as a target for pharmacological intervention. Overall, accumulating evidence has indicated that ABCA2 expression and subsequent endolysosomal compartmentalization of sterols is directly linked to cancer drug resistance [14,19,50] and may be an important regulator to maintain homeostatic levels of cholesterol for cellular function, growth and membrane integrity [14,51]. Recently, the specific properties of lysosomes within cancer cells were considered to provide a novel strategy for targeting of cancer chemotherapeutic agents [52]. Perhaps a plausible role for ABC transporters as functional members of dynamic protein complexes (rather than simple substrate transporters) in the processes of cell growth, differentiation and death has, thus far, been overlooked.

3.2. Cancer drug resistance

Adaptation of cancer cells to a single drug can render pleiotropic cellular effects and result in specific or general acquired resistance. The latter is appropriately termed multidrug resistance (MDR). The current strategy for cancer chemotherapy involves cocktails of pharmacological agents and MDR may be a cause of treatment failure. A number of ABC transporters have been implicated in cellular drug resistance [53]. In particular, members of the MDR and MRP transporter sub-families have been linked to simultaneous resistance to multiple cytotoxic drugs in cancer cells. MDR1 confers resistance to a variety of hydrophobic, amphipathic natural product drugs [11] whereas members of the MRP-sub-family are associated with resistance to anionic and neutral drugs frequently conjugated to acidic ligands [54]. Although several mechanisms can contribute to MDR, alterations in drug accumulation appears to be common both in cell culture and in model organisms [55]. The initial discovery linking membrane transporters with drug resistance came from an observation that MDR1 decreased the accumulation and toxic effects of various and structurally unrelated anti-neoplastic drugs in CHO cells [56,57]. Although the crystal structure of a human ABC transporter has yet to be resolved, the insights into putative structure and function of ABC transporters has been deduced from similarity with *E. coli*

homologs, MsbA and BtuCD [58,59]. The function of MDR1 in drug efflux is generally ATP-dependent, similar to homologous bacterial ABC transporters. However, the mechanism(s) of ATP hydrolysis-coupled substrate transport remains theoretical [60].

Extending the drug resistance paradigm to the A sub-family, an ovarian carcinoma cell line (SKEM) with acquired resistance to the estradiol-based agent, EM, was found to have a gene amplification at 9q34 and contain an ABCA2 amplicon [51]. EM is a synthetic conjugate of nitrogen mustard and estradiol with an antimitotic activity [61]. A homogenously staining region, typical for gene amplification, was found in chromosome 9q34, and in situ hybridization with a specific probe indicated a approximately sixfold amplification of the ABCA2 gene. The approximately fivefold increase in gene expression in SKEM cells was accompanied by an increased rate of dansylated EM efflux and, hence, drug resistance. Cells were sensitized to EM upon treatment with antisense ABCA2 RNA [51]. Similarly, ABCA2 overexpressing HEK293 cells (HEK293-ABCA2) were also shown to be EM-resistant [14]. Somewhat lower levels of resistance (approximately threefold) were observed for the same cells upon exposure to a structurally similar compound, estradiol. Supporting the observation that ABCA2 contributes to EM and estradiol resistance was that cells transfected with a putative dominant-negative mutant of ABCA2 have virtually no differences in toxicity compared to mock-transfected cells [19]. This study also showed that HEK293-ABCA2 cells were not resistant to agents that are structurally dissimilar to EM (melphalan, mitoxantrone, cisplatinum, taxol, and vinblastine) with the exception of a slight doxorubicin resistance. Further studies showed that HEK293-ABCA2 cells are also resistant to a free radical initiator, AAPH [44]. Although AAPH is unrelated to other sterol-related substrates, free radical or reactive oxygen species (ROS) induced damage may damage sterols, lipids and lipoproteins where cell survival may be facilitated by sequesteration of damaged molecules into the endolysosomal compartment. Even though ABCA2 overexpression did not confer mitoxantrone resistance in HEK293 cells, this transporter was shown to be up-regulated in a mitoxantrone-resistant small cell lung cancer cell line, GLC4-MITO [50]. The same cell line was approximately twofold more resistant to EM compared to parental GLC4 cells. Exposure of GLC4-MITO cells to both EM and mitoxantrone increased cellular accumulation of the latter, indicating an ability of EM to block efflux of mitoxantrone. Taken together, these results indicate that molecules with steroid-like structures are putative substrates for transport by ABCA2 and may cause induced expression. It is not known, however, whether such compounds interact directly with ABCA2 for transport across intracellular membranes, or if they serve a signaling molecule to initiate some form of transport cascade.

4. Conclusions

Several factors serve to limit progress in elucidating protein function for membrane-bound ABC transporters. These include structural biology studies that use traditional crystallization methods. Determination of protein–protein interactions of full-length, native proteins is hampered by their multiple domain structures and high molecular weight. Also, because of possible functional redundancy the high degree of homology among ABC transporters makes loss-of function studies difficult to interpret.

Although the structural and functional characterization of the ABCA2 transporter is in its infancy, there are several promising indications for future studies. The consequence of deregulated trafficking of cholesterol and sterol-related compounds at the cell and organism level, has effects on formation of arterial plaques and increases the risk for heart disease, AD and cancer. Links with the cellular response to ROS and elevated LDL cholesterol [62–65] make the fact that ABCA2 is abundantly expressed in macrophages [17], more pertinent to these pathologies. These connections and the identification of other cholesterol-responsive transporters from the same family (ABCA1 and ABCA7) may provide the platform for small molecule screening strategies for these novel targets. Impending studies could provide a wealth of information for the possibility of intercession in transport function as a conduit to pharmacological intervention in these diseases.

Abbreviations:

AAPH	2,2′-azobis-(2-amidinopropane)
Aβ	β-amyloid peptide
ABC	ATP-binding cassette
AD	Alzheimer’s disease
ADGE	amplified differential gene expression
ALD	X-linked adrenoleukodystrophy
APP	β-amyloid precursor protein
CF	cystic fibrosis
CNS	central nervous system
EGR-1	early growth response-1
EM	estramustine
ER	endoplasmic reticulum
FHC	Familial Hypercholesterolemia
HDL	high density lipoproteins
HMGCoA S	3-hydroxy-3-methylglutaryl CoA synthase
LA	linoleic acid
LAMP	lysosome-associated membrane proteins
LDL	low-density lipoprotein
LDLR	LDL receptor
LXR	liver X receptor

MDR1/Pgp1	multidrug transporter/P-glycoprotein
MRP1	multidrug resistance associated protein
NPC1	Niemann-Pick type C1
ROS	reactive oxygen species
SNP	single-nucleotide polymorphisms
SREBP2	sterol-response element binding protein-2

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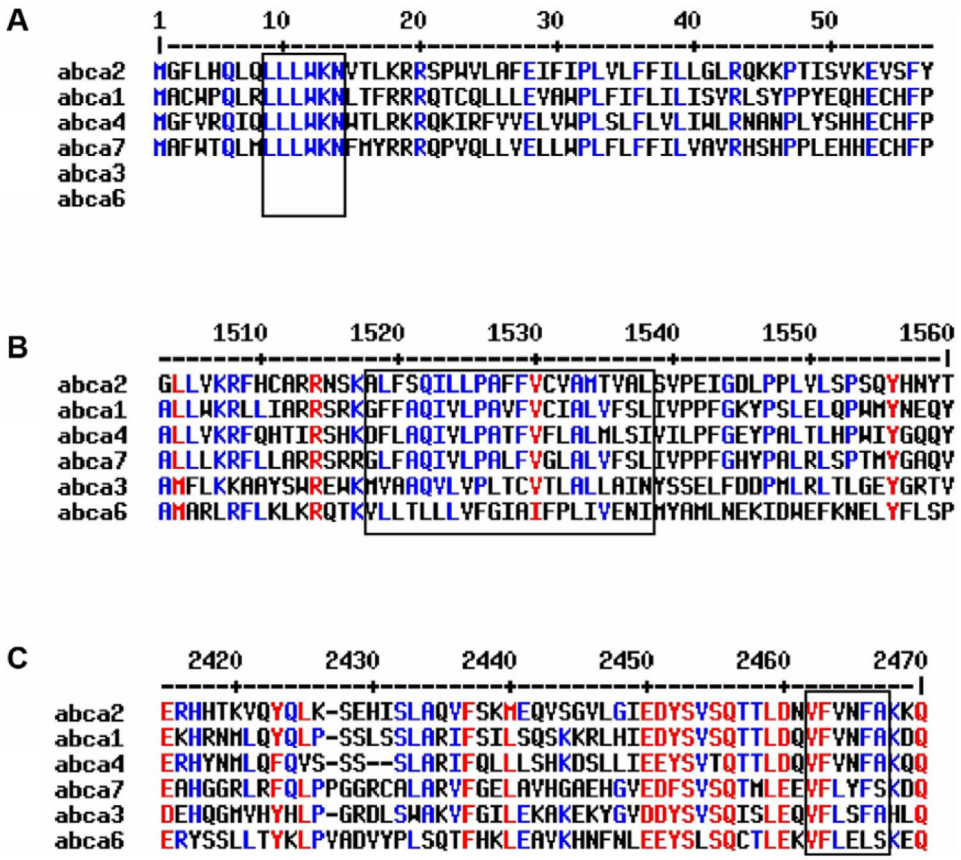

Fig. 1.

Alignment of ABCA transporters with homology to ABCA2; A: conserved motif at the beginning of the N-terminus with unknown function, B: high hydrophobic domain, C: highly conserved domain at the end of the C-terminus.

Table 1

Members of sub-family A of ABC transporter proteins

Member	Chromosome	Expression	Function	Pathology
A1	9q22-q31	Ubiquitous	Cellular lipid efflux	Tangier disease
A2	9q34	Brain	Cholesterol homeostasis	Alzheimer's disease
A3	16p13.3	Lung alveolar type II cells	Lipid transport	Newborn surfactant deficiency
A4	1p21	Retina	Flippase	Stargardt disease
A7	19	Myelolymphatic tissue, spleen	Cellular lipid efflux	Sjorgren syndrome
A12	2q34	Keratinocytes	Lipid traffic	Laellar ichthyosis type II
A13	7q12.3	Kidney, skeletal muscle	Unknown	Unknown