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Research Paper

RT-PCR analysis of ABC, SLC and SLCO drug transporters in human lung epithelial cell models

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Abstract

Objectives Carrier-mediated transport mechanisms play crucial roles in drug absorption and elimination processes, as well as in the transport of endogenous molecules affecting cellular regulation and function. In this study we used RT-PCR analysis to characterise the mRNA transcript expression of a wide range of membrane carrier transporters in several in-vitro lung epithelial cell models. Transporters studied included: 11 ATP-binding cassette (ABC) transporters, 11 solute carrier (SLC) transporters and 9 solute carrier organic anion (SLCO) transporters.

Methods The cell culture models included both established cell lines (A549, Calu-3, 16HBE14o-, BEAS-2B) and freshly isolated lung epithelial cells in primary culture (human bronchial and alveolar epithelial cells).

Key findings The expression profiles of several clinically relevant drug transporters were characterised using RT-PCR analysis. Our results showed differential transporter expression in cell culture models from different regions of the lung and also highlighted disparities when comparing lung cell lines with primary cell culture models. Differences in transporter expression between cell models of pulmonary and gastrointestinal origin were also noted.

Conclusions The information will guide and validate the use of in-vitro lung epithelial cell lines in the study of pulmonary administered drugs and candidate molecules.

Keywords alveolar epithelial cells; bronchial epithelial cells; drug disposition; drug transporters; pulmonary drug delivery

Introduction

The phylogeny, molecular biology and functional characteristics of membrane transporters have been major focuses in biopharmaceutical and cell physiology research over the last 15 years. Particular attention has addressed transporter expression within biological barriers such as the blood–brain barrier, liver, kidney and intestine.[1] Non-invasive drug delivery via the pulmonary route continues to represent an attractive avenue for many clinically relevant compounds. However, little is known about the expression of membrane transporters within lung epithelium, and even less about regional differences that may exist within the different lung epithelial surfaces (e.g. alveolar vs bronchial). Comparatively little is known about the potential impact of lung epithelial membrane transporters on the absorption and disposition of inhaled drugs. Active transport mechanisms within the respiratory tract also have the potential to modify the biodistribution of therapeutic drugs and environmental xenobiotics that enter the body by other routes such as the gastrointestinal tract, which may alter the retention or accumulation within the lung and ultimately result in toxicity.[2] Indeed, the recent work of Francombe and colleagues using an isolated perfused intact rat lung demonstrated the impact of an efflux transporter (mdr-11/P-glycoprotein (P-gp)) on the absorption of a model P-gp substrate when instilled into the airways.[3] However, it is clear that the impact of a transporter such as P-gp on the absorption and disposition of given substrate will also depend on the substrate's physicochemical properties such as its overall permeability profile (i.e. high or low permeability drug).

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Membrane transporters relevant to xenobiotic transport comprise three main groups: the ATP-binding cassette (ABC) transporters, of which there are approximately 50, the solute carrier (SLC) transporters, of which there are approximately 300, and organic anion transporting polypeptides, classified within the OATP/SLCO family, of which 36 members have been identified so far in human, rat and mouse.

ABC transporters are transmembrane proteins that use the energy of ATP hydrolysis to actively transport a wide variety of substrates across extra- and intracellular membranes.[4] Substrates include physiological metabolic products, lipids, sterols and a wide range of drugs.[5] Abnormalities in ABC transporter function have been implicated in inherited human diseases and in tumour and bacterial multidrug resistance. ABC transporter family members that are of particular significance with respect to drug absorption and disposition include: MDR1/P-gp, the multidrug resistance-related proteins (MRP1–9) and breast-cancer-related protein (BCRP).[6–8]

The SLC superfamily of transporters is organised into 47 families.[1,9,10] Solutes transported by the various SLC group members are extraordinarily diverse and include charged and uncharged organic molecules, inorganic ions, di- and tripeptides, and a variety of structurally related drugs, including prodrugs.[1] Both uptake and efflux SLCs influence physiological and pathophysiological function as well as the absorption and disposition of xenobiotics.[1] The SLC superfamily includes the organic cation transporters (OCT) and organic anion transporters (OAT) (SLC22 family), which play critical roles in renal and hepatic transport and the detoxification of a wide variety of compounds, including drugs, toxins, hormones and neurotransmitter metabolites.[11]

In 2004, Hagenbuch and Meier[12] reclassified the OATPs, formerly known as the SLC21 family. The SLCO family represents a phylogenetically based species-independent and open-ended nomenclature and classification system. These transporters are present in a variety of biological barriers and have a marked impact on xenobiotic absorption.

In this study we have examined the expression of mRNA transcripts in several lung epithelial cell types, including cells of bronchial and alveolar epithelial origin, primary cells and cell lines. The selection of transporters investigated in our study was based on their reported relevance in affecting drug disposition. Transporter proteins examined were: the ABC transporters MDR1/P-gp, MRP1–8, WHITE1 and BCRP; the SLC transporters OCT1–3, OCTN1 and -2, PEPT1 and -2 and OAT1–4; and the SLCO transporters OATP1A2, -1B1, -1B3, -1C1, -2A1, -2B1, -3A1, -4A1 and -5A1.

Materials and Methods

Cell culture

Human A549 cells

A549 cells are derived from a human pulmonary adenocarcinoma.[13] This cell line is widely used in studies of alveolar epithelium function because of its phenotypic similarity to alveolar type II (ATII) epithelial cells.[14]

Human A549 cells (ATCC CL-185 LGC Promochem) were obtained from the American Type Culture Collection (ATCC; Teddington, UK). Cells were seeded into six-well plates (Corning, Schiphol, the Netherlands) at a density of 40 000 cells/cm^2, and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin. The medium was replaced every 48 h. Monolayers reached confluence by day 5 of culture, at which time RNA was extracted.

Human Calu-3 cell line

This is a human bronchial epithelial cell line derived from an adenocarcinoma of the lung. Cells were obtained from the ATCC (ATCC HTB-55) and were seeded into six-well plates (Corning) at a density of 75 000 cells/cm^2 and cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, which was replaced every 48 h. Cells reached confluence by day 8 of culture, at which time either RNA was extracted or cells remained in culture for a further 7 days to allow for further differentiation[14] and subsequent RNA extraction.

Human 16HBE14o- cell line

This cell line was obtained from Dr Dieter C. Gruenert (California Pacific Medical Center, San Francisco, CA, USA). The cell line was generated by transformation of normal bronchial epithelial cells obtained from a 1-year-old male heart–lung transplant patient. Transformation was accomplished with SV40 large T antigen.[15] Cells were cultured at 100 000 cells/cm^2 in Eagle's minimum essential medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, 1 mM sodium pyruvate and non-essential amino acids, which was replaced every 48 h. Cell monolayer confluence was reached at 3 days. RNA was isolated after 11 and 18 days.

Human BEAS-2B cells

These are cells of the normal human epithelial cell line, immortalised using a hybrid of adenovirus 12 and SV40.[16] Cells were obtained from the ATCC (ATCC CRL-9609) and were seeded at a density of 100 000 cells/cm^2 and maintained in culture for 8 days before RNA was harvested. The cells were grown in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, which was replaced every 48 h.

Human alveolar epithelial cells (hAEpC)

Fresh human ATII epithelial cells (hAEpC) were isolated from non-tumour lung tissue obtained from patients undergoing lung resection. The use of human material for isolation of primary cells was reviewed and approved by the local ethics committee (Saarland State Medical Board, Germany). Isolation of primary human type II pneumocytes was performed according to a protocol modified from those of Ehrhardt and colleagues.[17] Briefly, finely minced lung tissue was digested for 40 min at 37°C using a combination of 150 mg trypsin type I (Sigma, Seelze, Germany) and 0.641 mg elastase (CellSystems, St Katharinen, Germany) in 30 ml HEPES-buffered balanced salt solution (137 mM NaCl, 5.0 mM KCl, 0.7 mM $Na_2HPO_4{\cdot}7H_2O$, 10 mM

HEPES, 5.5 mM glucose; pH 7.4) supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin. The ATII population was purified from the crude cell mixture using a combination of differential cell attachment, centrifugation with a percoll density gradient, and cell sorting with magnetic beads (anti-HEA (EpCAM) MicroBeads, Miltenyi Biotec, Bergisch Gladbach, Germany). The average yield of ATII cells was 0.8×10^6 cells/g tissue (n = 19) with a purity of >90% determined by staining cells for alkaline phosphatase. Purified ATII cells were either used directly for RNA isolation or seeded at a density of 600 000 cells/cm^2 on collagen/fibronectin coated Transwell inserts (Corning) using small airways growth medium (Lonza, Verviers, Belgium) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml) and 1% FBS. Under these culture conditions, the type II pneumocytes transdifferentiated into monolayers of type-I-like phenotype[18,19] at approximately day 8 in culture. RNA was extracted from freshly isolated ATII cells and from type-I-like phenotypes on day 8.

Human bronchial epithelial cells (hBEpC)

These primary/first-passage normal human bronchial epithelial cells were obtained from TCS CellWorks (ZHC-1101; Buckingham, UK) and cultured at an initial density of 10 000 cells/cm^2 for 10 days in bronchial epithelial growth medium (TCS CellWorks). The medium was replaced every 48 h. RNA was extracted on day 10 of culture.

Human colonic adenocarcinoma cells (Caco-2)

Cells were obtained from the European Collection of Animal Cell Cultures (ECACC, Salisbury, UK) and used as a reference cell line for expression studies.[20] The cells were cultured in six-well plates in DMEM supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin, replaced every 48 h. RNA was harvested on day 21 after seeding.

RNA isolation and RT-PCR

Selected drug transporter mRNA sequences were aligned with BLAST2.[21] Single exon regions were identified through use of SNPper.[22] Exons present in all transporter gene transcription variants and of suitable size (150bp+) were validated using Primer3[23] and primers were designed against these sequences (see Table 1). The polymerase chain reaction (PCR) was performed using cells from 3–5 different isolations. Briefly, total RNA was isolated and purified from cells using an RNeasy mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Reverse transcription was carried out using M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA), except for hAEpC, for which we used an Omniscript kit (Qiagen). The cDNA was initially amplified with Pd(N)$_6$ random hexamers (Amersham, Chalfont St Giles, UK). cDNA samples (corresponding to 40 ng RNA) were used for each PCR run with a Biotaq Core kit (Bioline, London, UK) including 0.75 μl 25 mM $MgCl_2$, 1 μl 2.5 mM dNTP mix, 1.25 μl Taq buffer, 0.1 μl Taq polymerase, 10 pmol primer (forward and reverse) and diethylpyrocarbonate-treated water up to a volume of 12.5 μl. The reaction was incubated at 94°C for 5 min, followed by thermal cycling: 35 cycles of 30 s at 94°C, 45 s at 54–60°C and 45 s at 72°C. After the last cycle, an additional step of 10 min at 72°C with the Taq polymerase completed any unfinished regions. DNA fragments were separated using gel electrophoresis (2% agarose) and were visualised using ethidium bromide staining. A HyperLadder IV size marker (Bioline) was run in parallel. Electrophoresis was carried out at 90 V for 45 min.

Results

The expression profiles for selected transporters in human respiratory epithelial cell models derived using semi-quantitative RT-PCR are shown in Table 2 (ABC transporters), Table 3 (SLC transporters) and Table 4 (SCLO transporters). β-Actin served as a positive control in all the RT-PCR reactions; negative controls comprised cDNA-negative, RT-negative wells. Levels of β-actin were consistent (+++) throughout all the different mRNA samples analysed. For reference we also summarise data obtained for the human colonic adenocarcinoma cell line, Caco-2. Several drug transporters have recently been investigated in Caco-2 cells using q-PCR technology.[24,25]

Figure 1 illustrates the typical signal intensities corresponding to the assigned mRNA expression index. The data from Bleasby and colleagues[26] refer to a microarray study of gene expression intensity in human whole lung tissue based on a reference set of 19 000 genes.

Discussion

In this study, we sought to generate a comprehensive mRNA profile for key drug transporters expressed in human lung epithelial cell types.

Expression analysis of ABC transporters

Table 2 summarises the expression of 10 ABC transporter genes in human respiratory epithelial cell models. MDR1/P-gp is probably the most commonly investigated ABC transporter in terms of drug absorption and disposition and is expressed in a variety of biological barriers and organs, including the blood–brain barrier, intestine, kidney and liver.[27] Within the lung, previous work has localised MDR1 to the bronchial and alveolar epithelial cells.[28–30] MDR1 functionality has also been demonstrated in lung epithelial continuous cell lines,[31,32] as well as in rat and human primary alveolar cells.[28,30] Our results for MDR1 confirm and complement the above and extend the knowledge of MDR1 expression to a range of human respiratory epithelial cell models commonly used in various in-vitro cell culture assays. The primary bronchial epithelial model, hBEpC, arguably a closer phenotype to the in-vivo cell type in terms of its primary cell nature, showed a low MDR1 signal. The 16HBE14o- cell line also displayed low levels of MDR1 expression, independent of time in culture. Higher MDR1 levels were detected in the widely used Calu-3 cells, which showed a temporal dependence, increasing with time in culture. Little to no expression of MDR1 was found in the BEAS-2B cell line. Within the limits of the semi-quantitative approach described here, the bronchial Calu-3 cells displayed the highest MDR1 transcript expression, which was

Table 1 Specific primer sequences for RT-PCR analysis

Transporter	Gene	Forward primer (5′ to 3′)	Reverse primer (3′ to 5′)	Product size (bp)	GenBank accession no.
MDR1	ABCB2	CAC CTG CAT TGT GAT TGC TC	AGA GTT CAC TGG CGC TTT GT	174	NM_000593
MRP1	ABCC1	GCT GTG AAG ACC CAG GAG AG	ACA GAC TTC GGC TGG AGA GA	230	NM_004996
MRP2	ABCC2	CTA TCC AAC TTG GCC AGG AA	AGG GTC CCA ACT CTC TCC AT	166	NM_000392
MRP3	ABCC3	GCA CTG CTG CAC AAC AAG AT	GGC GTT GAA GAA GGA ATT GA	157	NM_003786
MRP4	ABCC4	GAG GGA TGA ATT TGG CTT CA	ACA CCC TCT CAA TGG CTG AG	180	NM_005845
MRP5	ABCC5	AAC CAG CCA GTC CTC ACA TC	CCT TCT TCC TCT TCG GGA CT	247	NM_005688
MRP6	ABCC6	CAC CTG CTA AAC CGC TTC TC	CTG AAA CCC AGC GTA GAG GA	180	NG_007558
MRP7	ABCC10	CTG CCC TTC ATC CTC AAC AT	CGG CCA GAT GGC TAT ACA GT	211	NM_033450.2
MRP8	ABCC11	GCC ACA GCC ACT TCT TCA CT	GCC TAT TCC AGG GTT TCC AT	197	NM_145186.2
WHITE1	ABCG1	ATG GCT TAG ACC GGG AAG AT	GTT TCC TGG CAT TCA GGT GT	218	NM_016818
BCRP	ABCG2	GTG GCC TTG GCT TGT ATG AT	GAT GGC AAG GGA ACA GAA AA	180	NM_004827
PEPT1	SLC15A1	CCA TCA TGG CTC GGT TCT AT	ATC CAA TGG AGT GTC CTG CT	219	NM_005073
PEPT2	SLC15A2	CCA GCA ACA CTG CAC AAG TT	CCA GCA ACA CTG CAC AAG TT	222	NM_021082
OCT1	SLC22A1	CTG AGG GAG ACA TTG CAC CT	CGA CAG CAG GCA TAA GAT GA	161	NM_003057
OCT2	SLC22A2	ATC TAC GTG GGC ATC GTC TT	GTT CCA GTC CAC CTC GTA GC	186	NM_003058
OCT3	SLC22A3	GTC ACC TTC GCC TTC CTC TT	CAG CTG AGA GCG CTA GTG G	242	NM_021977
OCTN1	SLC22A4	CTG AGA ACG CTG TCA TCA CC	GCC AGG AAC ACG ACT GAC AT	182	NM_003059
OCTN2	SLC22A5	CTG TCC TCC GTG TTC CTG AT	AGA CAG CTC TCC TGC TCC AG	233	NM_003060
OAT1	SLC22A6	CAG CAA CAA GAG CAC CAG AA	TGG GTC ACC ATT TCC TCT TC	166	NM_004790
OAT2	SLC22A7	TGC TGC TAC CAC TGC ACT TC	ACT GTG GCA GGT TCA TCC TC	247	NM_006672
OAT3	SLC22A8	TGA CCT TCT CGG AGA TCC TG	ACC TCT CAG GCT TCC CAT TT	216	NM_004254
OAT4	SLC22A9	AGC TCT GTT CAT GGC GTT CT	GGG TCA TGT TTG TGG AAA CC	206	NM_080866
OATP1A2	SLCO1A2	TGG CTT TCT GAT TTG TGC AG	TCC TTC TTG ACC TCT TCT TTT TG	150	NM_134431
OATP1B1	SLCO1B1	AGG GTC TAC TTG GGC TTG TCT	CCA GCA GAA GGG ACA AAA TG	186	NM_006446
OATP1B3	SLCO1B3	AGA TAC CAA GGC ATC GGA CA	GCA ATG TTA GTT GGC AGC AG	154	NM_019844
OATP1C1	SLCO1C1	TGG GCA CAG TGT CAA TTC TC	TGG CCA GTA GTT GGG TTG TA	173	NM_017435
OATP2A1	SLCO2A1	AGG GGT GAA AGG AAG AAG GA	TTC TCA GTC CCT GCT CTG GT	244	NM_005630
OATP2B1	SLCO2B1	GGT AGG GAG GGA GAC TCA GG	CAG GCA CCC AGG AGA AAA TA	183	NM_007256
OATP3A1	SLCO3A1	AAA TCC TTC GCC TTC ATC CT	CCA CTC ATG GTC TTC CAG GT	207	NM_013272
OATP4A1	SLCO4A1	GGC ATC CTG TTC TTC CTG TG	CCG AAG TAG CTG ACG AAG GT	184	NM_016354
OATP5A1	SLCO5A1	TGG ACC TCA GCA AAA CCT TC	GGG CGA GAT GAA GTG AGG TA	233	NM_030958
β-actin	ACTB	AAA CTG GAA CGG TGA AGG TG	AGA GAA GTG GGG TGG CTT TT	171	NM_001101

All primers were derived from GenBank, using the accession numbers displayed. Where more than one transcript variant exists, accession number for variant 1 is displayed. Other variants can be accessed through this record.

Table 2 RT-PCR expression profiles of selected ATP-binding cassette (ABC) transporters in a variety of cell culture models including both primary and immortalised cell lines

	hBEpC	Calu-3 day 8	Calu-3 day 15	16HBE14o- day 11	16HBE14o- day 18	BEAS-2B	ATII	ATI-like	A549	Caco-2	Bleasby *et al.* 2006
MDR1	+	++	+++	+	+	–	++	+	+	+++	–
MRP1	+++	+++	+++	++	+++	+++	+++	+++	+++	++	++
MRP2	++	++	++	++	++	++	++	+	+++	+++	–
MRP3	++	+++	+++	++	++	++	+++	+++	+++	+++	++
MRP4	++	+	–	–	+	–	++	++	+	+	+
MRP5	+++	++	+++	+	++	+++	++	++	++	++	++
MRP6	+	+	++	+	++	++	+++	+++	+	+++	+
MRP7	++	++	+++	++	+++	+++	++	+++	++	++	+++
MRP8	+	–	+	–	–	–	++	++	–	–	++
WHITE1	+++	+	++	+++	+++	++	+++	+++	+	++	N/A
BCRP	++	+	+	+++	+++	+++	++	++	+++	+++	++
β-Actin	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

Signal intensity (see Figure 1): –, no expression; +, low expression; ++, moderate expression; +++, high expression. The final column refers to the gene expression intensity in human whole lung tissue based on a reference set of 19 000 genes.[26] Symbols represent intensity of gene expression within: –, 0–25% quartile of genes examined; +, 25–50% quartile; ++, 50–75% quartile; +++, 75–100% quartile.

comparable to that seen in Caco-2 cells. In the intact human (and rat) lung, quite intense MDR1 staining can be seen in bronchial epithelium.[28] Earlier reports have found significantly greater P-gp functionality in Caco-2 cells (as measured by rhodamine123 transport) compared with 16HBE14o-[32] and indeed Calu-3 cells.[31]

The epithelial type II cells isolated from human lung were studied as freshly isolated cells (ATII) and were also cultivated for 8 days (ATI-like), at which point they adopt many of the characteristics of type-I alveolar epithelial cells.[18,19] The human ATII cells in this work showed moderate expression of MDR1, with the ATI-like phenotype showing a lower MDR1 signal, similar to that for the adenocarcinoma cell line, A549. Only the human ATII cells showed comparable levels of MDR1 transcript to the Caco-2 cells. The culture of primary human alveolar epithelial cells is highly specialised, and clearly more functional studies on MDR1 transport in this unique human model system are needed. Immunohistochemistry has shown significant P-gp staining across the entire alveolar epithelial surface of intact human[28,30] and rat[28] lung. (Note that type-I epithelium comprises approximately 95% of the total alveolar epithelial surface area.) Functionally, human ATI-like cells have been shown to display significantly less P-gp activity than Caco-2 cells.[30] In the rat, mdr1a and mdr1b mRNA levels have been reported to increase as primary ATII cells progress to an ATI-like phenotype.[28] Furthermore, the rat ATI-like cells displayed greater P-gp protein and functionality than A549 cells but lower expression and functionality than Caco-2 cells. Recently published studies using an isolated perfused intact rat lung[3] and mdr1a-deficient mice[33] have given rise to conflicting conclusions on the significance of P-gp on the absorption of a P-gp substrate instilled into the airways. Clearly the permeability characteristics of substrates (intrinsically high or low permeability) will have a bearing on the overall impact of P-gp on a substrate's absorption profile.

Table 3 RT-PCR expression profile of selected solute carrier (SLC) transporters of relevance to drug disposition

	hBEpC	Calu-3 day 8	Calu-3 day 15	16HBE14o- day 11	16HBE14o- day 18	BEAS-2B	A549	Caco-2	Bleasby *et al.* 2006
PEPT1	–	+	++	+	++	+	+	+++	–
PEPT2	++	+	++	+	++	++	+	+++	++
OCT1	+	+	+	+	+	++	+	+	+
OCT2	–	–	–	–	–	–	–	+	–
OCT3	++	+++	++	–	–	–	+++	+	+
OCTN1	–	++	++	+	++	+++	++	++	++
OCTN2	+	+	+	+	++	+	+	++	++
OAT1	–	–	–	–	–	–	–	+	–
OAT2	–	–	–	–	–	–	–	+++	++
OAT3	–	–	–	–	–	–	–	++	–
OAT4	–	+	++	++	++	++	++	++	–
β-Actin	+++	+++	+++	+++	+++	+++	+++	+++	+++

Signal intensity (see Figure 1): –, no expression; +, low expression; ++, moderate expression; +++, high expression. The final column refers to the gene expression intensity in human whole lung tissue based on a reference set of 19 000 genes.[26] Symbols represent intensity of gene expression within: –, 0–25% quartile of genes examined; +, 25–50% quartile; ++, 50–75% quartile; +++, 75–100% quartile.

Table 4 RT-PCR expression profile of selected solute carrier organic anion (SLCO) transporters

	hBEpC	Calu-3 day 8	Calu-3 day 15	16HBE14o- day 11	16HBE14o- day 18	BEAS-2B	A549	Caco-2	Bleasby *et al.* 2006
OATP1A2	++	–	++	–	–	+	++	+++	–
OATP1B1	–	+++	+++	–	–	–	+	–	–
OATP1B3	–	++	+++	–	–	+	+	–	–
OATP1C1	–	++	++	–	–	–	+	–	–
OATP2A1	–	–	–	–	–	–	–	+++	+++
OATP2B1	–	+++	+++	–	–	–	+++	+++	++
OATP3A1	+++	+++	+++	++	++	++	+++	–	++
OATP4A1	++	++	++	++	++	+++	++	++	++
OATP5A1	–	+	–	–	–	+	+	–	–
β-Actin	+++	+++	+++	+++	+++	+++	+++	+++	+++

Signal intensity (see Figure 1): –, no expression; +, low expression; ++, moderate expression; +++, high expression. The final column refers to the gene expression intensity in human whole lung tissue based on a reference set of 19 000 genes.[26] Symbols represent intensity of gene expression within: –, 0–25% quartile of genes examined; +, 25–50% quartile; ++, 50–75% quartile; +++, 75–100% quartile.

Another family of ABC transporters is represented by the ABCC group to which MRP transporters belong. The different family members are variously expressed throughout the tissues of the body.[4] In particular, MRP1 has been quite extensively investigated for its role in drug resistance.[34] The transporter shares many similarities with MDR1, including substrate specificity and localisation, and has 15% sequence homology with this protein.[35] Significantly less work has been done to determine the profile of MRPs in the lung. MRP1 has been shown to be expressed in the lung, in both whole lung samples and cell cultures.[36,37] In the current study, the primary bronchial epithelial model, hBEpC, showed strong MRP1 and MRP5 transcript signals, moderate MRP2, MRP3, MRP4 and MRP7 signals and weak MRP6 and MRP8 signals. The only notable differences between the immortalised cells (16HBE14o- and BEAS-2B) or bronchial adenocarcinoma (Calu-3) cells compared with hBEpC were: low/complete absence of expression of MRP4 transcript in BEAS-2B, Calu-3 and 16HBE14o- cells; low levels of MRP5 transcript evident on day 11 in 16HBE14o- cells; no MRP8 transcript in the BEAS-2B, Calu-3 (day 8) and 16HBE14o- cells.

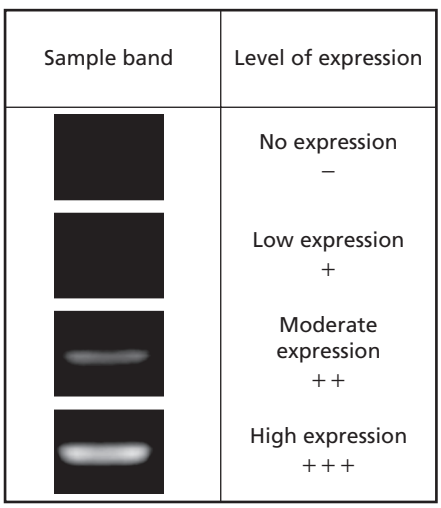

Figure 1 Representative samples of RT-PCR signal strengths. Data were grouped according to the intensity of the obtained signals of the product bands on agarose gels.

The ATII cells showed strong signals for MRP1, MRP3 and MRP6, with the remaining MRP transcripts showing moderate expression in the ATII model. The ATI-like phenotype showed a similar result to the ATII cells except for a lower MRP2 transcript and a higher MRP7 level. The adenocarcinoma cell line, A549, displayed higher MRP2 and lower MRP4, MRP6, MRP8 levels than both the ATII and ATI-like cells.

The G family of ABC transporters consists of half-transporters that oligomerise for function. Here we studied the expression of ABCG1 (WHITE1), involved in steroid and lipid transport, and ABCG2 (BCRP), a multidrug transporter which, like MDR1 and MRP family members, is constitutively expressed in tissue and is also able to confer drug resistance in cancer cells.[38] The primary hBEpC showed a strong WHITE1 signal which was similar to that seen in the 16HBE14o-cells; Calu-3 and BEAS-2B cells showed lower levels. The hBEpC showed moderate BCRP levels, with the Calu-3 displaying somewhat lower levels of expression and the 16HBE14o- and BEAS-2B cells higher levels. The ATII cells and the ATI-like phenotype showed strong signals for WHITE1 and moderate signals for BCRP. In comparison, the adenocarcinoma A549 cells showed low WHITE1 expression and high BCRP expression.

Expression analysis of SLC transporters

The expression of 11 SLC transporter genes in human respiratory epithelial cell models is summarised in Table 3. We chose the transporters that are most relevant to drug disposition. The organic cation and anion transporters are known to be expressed in organs of elimination, notably the kidney and the liver, and have also been reported in barriers such as the blood–brain barrier and intestine.[39,40] Transcripts for the organic anion transporters OAT1, OAT2 and OAT3 had little or no expression in any of the bronchial cell models, and were also negligible in the A549 cells. We were able to demonstrate positive expression for all of the SLC transporters studied in Caco-2 cells. OAT4 was absent or

showed low expression in the hBEpC but was present at low-to-moderate levels in the other bronchial cells models comprising transformed cells. OAT expression has been described in developmental studies in embryonic lung tissue,[41] but limited information is available on expression in the adult lung. In the microarray experiments of Bleasby and colleagues,[26] the OAT family members showed comparatively low levels of expression intensity in whole human lung tissue, with only OAT2 appearing within the 50–75% (++) quartile of gene expression intensity.

With regard to the organic cation transporters, our data showed OCT1 to be moderately expressed in BEAS-2B, whereas its expression was low in all other bronchial models and A549 cells. OCT2 showed little or no expression in any of the bronchial cell models, and was also absent from A549 cells. Again, expression in the Caco-2 cells serves as a positive control for this negative data. Expression of OCT3 showed a variable pattern across the bronchial cells, with moderate-to-high expression in the hBEpC and Calu-3 cells, and negligible expression in the 16HBE14o- and BEAS-2B models. OCT3 expression was pronounced in the alveolar A549 cell line. While OCTN1 was negligible in hBEpC, the expression of this transporter was evident at low to high levels in the other investigated bronchial cells. Finally, except for the day 18 16HBE14o- cells, the expression of OCTN2 was consistently low in all the respiratory cell cultures studied.

The OCT family members have previously been found at various expression levels in several lung tissue specimens and cell culture models. Ishiguro and colleagues[42] identified OCT3 protein expression in A549 cells and primary rat ATII cells. Wang and colleagues[43] reported OCT3, OCTN1 and OCTN2 protein expression in the A549 cell line, and Miakotina and colleagues[44] also showed robust expression of OCT2 protein in primary rat ATII cells. Lips and colleagues[45] used immunohistochemistry to demonstrate expression of OCT1, OCT2 and OCT3 in both human and rat lung ciliated airway epithelial cells of intact lung tissue. Similarly, Horvath and colleagues[46] used immunocytochemistry to show OCTN1 and OCTN2 protein expression in airway epithelial cells from intact human lung but did not confirm expression of OCT1, -2 and -3. In the microarray work of Bleasby and colleagues,[26] expression of OCTN1 and OCTN2 transcripts appeared to be in the 50–75% quartile of gene expression intensity, whereas OCT1 and OCT3 were in the 25–50% quartile and OCT2 even lower at 0–25%.

The expression of the proton-dependent peptide transporters (PEPT) in the kidney and intestine is well characterised.[47] PEPT1 expression within the intestine is well recognised, although its expression in the lung is less established. Here we showed that PEPT1 and PEPT2 transcripts were expressed at much higher levels in the Caco-2 cells than in any of the respiratory cell models. Bleasby and colleagues[26] reported absence of PEPT1 but presence of PEPT2 at the mRNA transcript level in whole human lung (25–50% quartile of gene expression intensity). In our studies, PEPT1 had low-to-moderate expression in all the bronchial cell lines, but negligible expression in the primary bronchial cell model, hBEpC. The importance of PEPT2 expression in kidney is recognised, as is its presence in lung. The protein has been localised in bronchial epithelial cells and to ATII by a number of research groups,[48–50] and we have shown expression in all our culture models. In our studies, PEPT2, similar to PEPT1, showed low-to-moderate expression in all the bronchial cells, with some evidence of temporal dependence in both Calu-3 and 16HBE14o- cells. The hBEpC and BEAS-2B cells showed a greater intensity of signal for PEPT2 than PEPT1. The hBEpC model showed agreement with the data of Bleasby and colleagues[26] for whole human lung.

Expression analysis of SLCO transporters

The OATPs are responsible for transport of a considerable number of endogenous solutes and xenobiotic compounds of diverse chemical nature. The expression of nine OATP transporter genes in human respiratory epithelial cell models is summarised in Table 4. We found OATP2A1 transcript to be completely absent from all bronchial cell models, as well from the A549 cell line; this transcript was expressed at high levels in Caco-2 cells. OATP5A1 was either absent or present only at very low levels in the bronchial and A549 cells, consistent with the finding of Bleasby and colleagues.[26] OATP3A1 and OATP4A1 transcripts were expressed at moderate-to-high levels in all the bronchial epithelial cell models as well as in the A549 cell line. Adachi and colleagues[51] and Tamai and colleagues[52] reported RNA transcript for OATP3A1 (OATP-D) in whole human lung, and also the presence of OATP4A1 (OATP-E).[52] The transcript for OATP1A2 (OATP-A) was expressed in hBEpC, Calu-3 (day 15), BEAS-2B and A549 cells. Kullak-Ublick and colleagues[53] reported the RNA transcript for OATP1A2 in whole human lung. Expression of OATP1B1, OATP1B3, OATP1C1, OATP2B1 transcripts was essentially limited to the Calu-3 cells among the bronchial models, with expression at moderate-to-high levels, while all other bronchial cells displayed low (BEAS-2B) or negligible expression. The presence of RNA transcript for OATP2B1 (OATP-B) has previously been demonstrated in whole human lung.[52,53] We found that OATP5A1 was absent in the bronchial cell models, except for Calu-3 at day 8 and BEAS-2B, where expression was low.

Conclusions

The impact of active carrier mechanisms on the absorption and disposition of xenobiotics is now clearly recognised, with an increasing awareness of the functional role that such carriers possess in the transport of endogenous molecules that serve as key modulators of cellular function. In-vitro cell-based models have a clear role in mechanistic studies of membrane transport and the biopharmaceutical screening of drug candidates.[54] In this study we sought to provide an analysis of key carrier transporters within a wide range of in-vitro respiratory cell culture models commonly used in biopharmaceutics and pharmacological research. Our results show differential transporter expression in cell culture models from different regions of the lung, and also highlighted disparities when comparing cell lines with primary cell culture models. Moreover, a number of variations in

transporter expression in cell models of pulmonary and gastrointestinal origin were discovered. The data provided will help guide the use of particular in-vitro respiratory cell models, and also partly explain potential differences in outcomes. Our investigation provides an mRNA expression profile of many prevalent drug transporters in several pulmonary cell models; a more extensive study on their functional impact is required.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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