The gene structure and expression of human ABHD1: overlapping polyadenylation signal sequence with Sec12

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Abstract

Background: Overlapping sense/antisense genes orientated in a tail-to-tail manner, often involving only the 3'UTRs, form the majority of gene pairs in mammalian genomes and can lead to the formation of double-stranded RNA that triggers the destruction of homologous mRNAs. Overlapping polyadenylation signal sequences have not been described previously.

Results: An instance of gene overlap has been found involving a shared single functional polyadenylation site. The genes involved are the human alpha/beta hydrolase domain containing gene 1 (ABHD1) and Sec12 genes. The nine exon human ABHD1 gene is located on chromosome 2p23.3 and encodes a 405-residue protein containing a catalytic triad analogous to that present in serine proteases. The Sec12 protein promotes efficient guanine nucleotide exchange on the Sar1 GTPase in the ER. Their sequences overlap for 42 bp in the 3'UTR in an antisense manner. Analysis by 3' RACE identified a single functional polyadenylation site, ATTAAA, within the 3'UTR of ABHD1 and a single polyadenylation signal, AATAAA, within the 3'UTR of Sec12. These polyadenylation signals overlap, sharing three bp. They are also conserved in mouse and rat.

Conclusions: Mammalian ABHD1 and Sec12 genes contain a conserved 42 bp overlap in their 3'UTR, and share a conserved TTTATTTAAA/TTTAATTAAA sequence that serves as a polyadenylation signal for both genes. No inverse correlation between the respective levels of ABHD1 and Sec12 RNA was found to indicate that any RNA interference occurred.

Background

Antisense RNA-mediated regulation is widespread in bacteria [1]. Recently, computational analysis of the human and mouse transcriptome identified many potential pairs of transcripts that overlap in an antisense fashion [2–5] indicating that the regulation of gene expression by antisense could be a more common phenomenon in mammalian cells than previously thought. The number of potential gene pairs identified range from 56–144 in humans [2–4] and 93–2,431 in mouse [4,5]. From an evolutionary standpoint, one would expect strong conservation of gene pairs between man and mouse paralleling the general conservation of mRNAs between these species. The large number of gene pairs that have been identified in the mouse suggests that many more are likely to be discovered by both in silico and gene expression methods in humans.
Gene overlap increases the likelihood that mRNAs derived from complementary genes will form double-stranded RNA when both genes are transcribed simultaneously. Cells monitor the quality of their mRNAs and degrade any transcripts that are incompletely translated [6]. RNA interference (RNAi) is the process by which double-stranded RNA triggers the destruction of homologous mRNAs. Considerable attention is now being given to the use of RNAi as a potential therapeutic tool [7]. The double-stranded RNA is cut into small RNAs by double strand-specific RNases. These small RNAs subsequently guide a protein nuclease to destroy their complementary mRNA targets in a catalytic manner [8,9] leading to a reduction in the expression of that particular protein.

The function of naturally occurring antisense RNAs in eukaryotes is beginning to be understood. However, no generalisations concerning the mechanism of action can be made based on those few gene pairs that have been examined experimentally. One possible function of these antisense RNAs is to control the post-transcriptional levels of their complementary RNA by regulating their stability [10,11]. Genes can overlap in many ways; they may overlap in their 5' or 3' regions or be nested. One or both of the paired transcripts may code for a protein. A common bi-directional promoter such as that between the alpha 1 and alpha 2 type IV collagen mRNAs regulates and co-ordinates the expression of these related genes [12]. The expression of the Surfeit locus genes, Surf-1 and Surf-2 are co-ordinated [13]. The reason for their co-ordinated expression remains to be determined since they are sequence-unrelated.

Both the histidyl-tRNA synthetase gene and the N-myc oncogene have overlapping gene partners arranged in a head-to-head manner. The histidyl-tRNA synthetase gene overlaps with an antisense gene, HO3, with which it shares extensive amino acid sequence homology [14]. It is unknown whether expression interference occurs, but they do have distinct tissue expression patterns. N-myc transcripts and its antisense partner, N-cym, form an RNA-RNA duplex and their expression is co-regulated, although the function of the antisense RNA is not yet understood [15]. The B cell maturation protein gene (BCMA) is the receptor for the tumour necrosis factor family member TALL-1. Nested within its mRNA is an antisense-BCMA transcript that is co-expressed [16].

The majority of gene pairs overlap in a tail-to-tail orientation, and often involve only the 3'UTRs [3,4]. The basic fibroblast growth factor gene (bFGF) has a tail-to-tail complementary transcript encoding GFG, which is a member of the MutT family of antimutator NTPases [17]. The resulting double-stranded RNA is a substrate for adenosine to inosine modification that would trigger rapid degradation of the reacting RNAs [11]. Such examples indicate that antisense RNA can exhibit both regulatory and coding capacities. Thymidylate synthase mRNA overlaps with the 3'UTR of an antisense transcript, rT$\alpha$sAlp$\alpha$, the expression of which was inversely correlated with the level of thymidylate synthase mRNA [18]. Thymidylate synthase mRNA is cleaved in a site-specific manner suggesting that it is down regulated through a natural RNA-based antisense mechanism. The thyroid hormone receptor gene, c-erbA$\alpha$alp$\alpha$, overlaps with RevErb in a tail-to-tail orientation [19]. The c-erbA$\alpha$alp$\alpha$ gene has two isoforms and there is evidence that the expression of isoform alpha2 is negatively regulated by antisense interactions with the complementary RevErb mRNA [20]. Antisense transcripts of the epithelial Na/Pi cotransporter have no effect on transcript stability, but Pi transport activity is reduced suggesting that they interfered with the translation of the transporter mRNA [21]. The human Misshappen/NIK-related kinase and the nicotinic cholinergic receptor, epsilon polypeptide 3'UTRs overlap [22]. They possess the classical AATAAA and ATTAAA polyadenylation signal sites respectively, but these sites do not overlap. The Surfeit locus contains another example of overlapping genes. The 3'UTRs of Surf-2 and Surf-4 overlap in mouse, but not man [23]. However, the murine polyadenylation signals do not overlap. The PR264/SC35 splicing factor is a member of the SR protein family. There are many varieties of antisense transcripts, entitled ET RNAs, which overlap predominantly in the 3'UTR of PR264/SC35, of which some may be protein-encoding [24]. Single cells co-expressed both mRNAs.

The research presented here is the case of two coding transcripts, human ABHD1 and Sec12, which overlap in their 3'UTRs. In the mouse, the presence of antisense transcripts of the lung alpha/beta hydrolase-1 (ABHD1) [25] and prolactin regulatory element binding (Sec12/PREB) genes [26] has been identified by a computational search [3]. Recently, we cloned three closely related cDNAs from a murine lung cDNA library [25], the open reading frames (ORF) of which contained a predicted alpha/beta hydrolase domain [27], containing a catalytic triad analogous to that present in serine proteases, leading them to be named lung alpha/beta hydrolase (LABH1-3) fold proteins. Subsequently, their tissue distribution suggested that they were more abundant in other tissues and, in keeping with the wishes of the gene nomenclature committee; they have been designated alpha/beta hydrolase domain containing genes 1, 2 and 3 (ABHD1, ABHD2 and ABHD3). The proteins encoded by their ORFs are related to the Escherichia coli ORF YHET that belongs to the prosite UPF0017 protein family, whose function remains unknown [28].
A BLAST search of the GenBank database with the cDNA sequence of human ABHD1 revealed a 3'UTR region of similarity with rat Sec12/PREB cDNA sequence [29]. In yeast, Sec12p is involved in vesicle budding from the endoplasmic reticulum (ER) and the formation of autophagosomes [30,31]. Sec12p is a type II transmembrane glycoprotein protein with a large cytosolic domain, which promotes efficient guanine nucleotide exchange on the Sar1 GTPase [30,32,33]. A recombinant part of rat Sec12, encompassing residues 175–417, has been reported as the ploactin regulatory element binding protein (PREB) transcription factor which regulates the activity of the ploactin promoter [29]. The murine PREB gene has been localised to the proximal end of chromosome 5 [26] and a partial human cDNA encoding PREB was cloned from brain and mapped to 2p23 [34]. Overall, Sec12/PREB transcripts are highly abundant in tissues that are active in secretion [29]. To investigate the nature of the shared cDNA sequences of human ABHD1 and Sec12, 3' RACE was used to clone the human homologue of murine ABHD1, the human and mouse Sec12 cDNA sequences and determine the location of the polyadenylation sites. I found that the human ABHD1 and Sec12 3'UTRs share overlapping polyadenylation signal sequences. Overlapping polyadenylation signal sequences have not been described previously in eukaryotic genomes.

Results

Human ABHD1 cDNA

The human homologue of the murine alpha/beta hydrolase domain gene 1 (ABHD1) was cloned by 3'RACE and sequenced. The human ABHD1 1331 bp cDNA sequence has a 33 bp 5'UTR, the first ATG of the ORF is in good sequence consensus for an initiation methionine, and has a short, 69 bp, 3'UTR which includes an ATTAAA polyadenylation signal at 1294–1299 (GenBank accession No. AY033290) (Fig. 1). The ORF encodes a 405 residue predicted protein with a 45,221 Da molecular mass and an isoelectric point 5.80.

Sequence comparison of the human ABHD1 protein with other members of the prosite UPF0017 family

The human ABHD1 cDNA was compared with that of the mouse [25]. They are of similar length, having 81% identity at the nucleotide level. However, there is a 15 bp deletion in the human sequence in the 5' region relative to the mouse sequence. In the mouse, this region contains an initiation ATG codon that is in excellent sequence context for the start of translation. Consequently, the human protein is eight residues shorter at the NH2 terminal and has the ORF starting at the second ATG site on the corresponding mouse sequence (Fig. 2). A database search identified a porcine EST (B1184779) and a rat EST (AW916573) that have predicted starts of translations that are the same as the human and mouse proteins respectively. Overall, the human and mouse proteins have 79% identity and 96% similarity and all 8 exon/exon boundaries are conserved. The human protein has an additional serine residue inserted close to the COOH terminal. A database search of the human genome with the human ABHD1 protein sequence identified two other related genes belonging to the prosite UPF0017 family. They are the HSI-2 protein [35] that is homologous to murine protein ABHD2 and the ORF of the unnamed cDNA (AI007152) [36]. The deduced protein sequence of this unnamed cDNA (AAC19155) has been truncated to the first methionine residue by homology with murine ABHD3 [25]. A comparison of these three proteins showed that human ABHD1 is most closely related to the AAC19155/ABHD3 protein having 43% identity and 76% similarity (Fig. 2). All three proteins have single predicted amino-terminus transmembrane domains despite having very little sequence identity in this region. The central cores of the three proteins are predicted to form alpha/beta hydrolase folds. This catalytic domain is found in a very wide range of enzymes such as serine proteases. In human ABHD1, the predicted catalytic triad is Ser203, Asp329 and His358 and is conserved in all members of the prosite UPF0017 family [25]. The suggested reaction mechanism is that the sidechain group of Ser203 acts as a nucleophilic centre; His358 sidechain acts as a general base and is hydrogen bonded to the carboxylic group of Asp329 to form a charge relay system. Human ABHD1, HSI-2/ABHD2 and protein AAC19155/ABHD3 are also expressed in lung tissue (Fig. 3).

The ABHD1 and Sec12 genes overlap in an antisense manner

Intriguingly, a database search of expressed sequences with the human ABHD1 cDNA sequence identified numerous human EST sequences with homology to the rat Sec12/PREB cDNA sequence [29]. To determine nature of this shared gene homology this region was amplified by PCR from human genomic DNA using primers corresponding to the ends of a human ABHD1/Sec12 electronic contiguous sequence. The sequence of this cloned ampli- con confirmed that ABHD1 and Sec12 genes overlap in an antisense manner (data not shown). Subsequently, the sequence of this region was found to match that of BAC clone RP11-195B17 (GenBank accession AC013403 Genome Sequencing Center, St. Louis, USA). To identify the extent of the gene overlap the human and murine Sec12 cDNAs were cloned by 3'RACE and sequenced and the location of their polyadenylation sites identified.

The human Sec12 2059 bp cDNA sequence has 131 bp 5'UTR; the first ATG of the ORF is in excellent sequence context for the start of translation and has a 677 bp 3'UTR which includes a single polyadenylation signal, AATAAA, at 2010–2015 (GenBank accession No. AF023687) (data...
Figure 1
The cDNA sequence and translation of human ABHD1. The exon/exon boundaries were determined by comparison with the sequence of genomic BAC clone RP11-195B17. An asterisk indicates the 3′a stop codon. The underlined nucleotide pairs indicate the exon/exon boundaries. There is a single polyadenylation signal (attaaa at 1294–1299) shown in bold type and underlined.
Figure 2

Sequence comparison of the human and murine ABHD1 proteins with two other closely related human ABHD proteins. The species are; Homo sapiens, Hs and Mus musculus, Mm. The proteins are human ABHD1; murine ABHD1 [25]; the putative transmembrane protein, HS1-2, ABHD2 [35] and the ORF of clones 23649 and 23755, accession No. AAC19155 truncated to first methionine residue, ABHD3 [36]. The stop codons are indicated by red asterisks. The predicted amino-terminus transmembrane domains are shown underlined. The predicted alpha/beta hydrolase fold domain is shown by a continuous blue line above the alignment and the predicted catalytic triads (which on human ABHD1 it is Ser203, Asp329 and His358) are indicated in bold and highlighted in green. The locations of the exon/exon boundaries are shown on the protein sequences as underlined residues. Residues conserved in all proteins are indicated by an (*), strongly conserved residues by (:) and weakly conserved residues by (.). Residues are colour coded: basic, DE, red; acidic, KR, pink; polar, CGHNQSTY, green and hydrophobic, AFILM-PVW, red.
not shown). The murine AATAAA polyadenylation signal at 1953–1958 (GenBank accession No. AF150808) (data not shown). The human ABHD1 and Sec12 cDNAs have a 42 bp overlap in their 3'UTR (Fig. 4). A conserved TTTAATAAAA/TITTAATAAAA sequence serves as a polyadenylation signal for both genes. The ABHD1 cDNAs have short 3'UTRs and use an ATTTAA polyadenylation signal, whilst the Sec12 cDNAs use an AATAAA polyadenylation signal. These signals are also conserved in mouse, rat, cow and pig ABHD1 and Sec12 ESTs sequences (Fig. 4). Also conserved are the sequences adjacent to the common ABHD1 and Sec12 polyadenylation sites suggesting that they may be important recognition sites for pre-mRNA cleavage factors. In the human and mouse ABHD1 mRNAs, the 3'UTR region upstream of the polyadenylation signals is more uracil rich (37–40%) than the expected 30% average for a 3'UTR [37] suggesting that this region may harbour upstream sequence elements involved in polyadenylation.

The cDNA sequence of the human Sec12/PREB gene has previously been reported [34]. However, nucleotide cytidine-186 is thymidine in their sequence and this alters the deduced amino acid sequence from a leucine to a phenylalanine at residue 19. Leucine-19 is conserved in mouse, rat and Caenorhabditis elegans Sec12/PREB proteins. The mouse Sec12/PREB cDNA sequence has been previously reported [26]. However, the ORF reported here differs by the inclusion of an inserted glycine-136 residue. Glycine-136 is conserved in the human and rat homologues [29,34].

**Human ABHD1 and Sec12 genes**

Previously, the human PREB gene was mapped to chromosome 2p23 [34]. A BLAST search of the human genome identified the location of the ABHD1 and Sec12 genes as 2p23.3, being encoded on opposite strands. They are flanked by the cell growth regulatory with EF-hand domain gene, CGR11 in the telomeric direction and by the NAS hypothetical protein, LOC165086, in the centromeric direction (Fig. 5A). Both are nine exon genes, the ABHD1 gene spans 6.9 kb and the Sec12 gene spans 3.8 kb (Fig. 5B). All splice donor/acceptor sites contained consensus GT/AT dinucleotides. Both ABHD1 and Sec12 have CpG islands in their promoter regions, such islands being one of the characteristics of housekeeping genes [38]. The ABHD1 gene has a short CpG island (264 bp, 72% GC) 5' of and encompassing part of exon 1 and the Sec12 gene has a longer CpG island (774 bp, 71% GC) spanning exon 1. The genes for HS1-2/ABHD2 and ABHD3 are much longer than ABHD1 and are located on different chromosomes. The 107 kb ABHD2 gene is located at 15q26.1 and the 54 kb ABHD3 gene is located at 18q11.1 close to the centromere.

**Tissue and cellular distribution of human ABHD1 and Sec12 mRNA**

The expression of the ABHD1 and Sec12 genes in different adult tissues relative to the expression of the housekeeping gene, β-actin, was examined by real time PCR (Fig. 6A). Both genes were expressed in all tissues examined, being highest in skeletal muscle, but the expression level of β-actin/μg cDNA was low in skeletal muscle relative to all the other tissues examined. The expression of ABHD1 in spleen was detectable by SYBR green fluorescence, but was below the level of detection by ethidium bromide agarose gel electrophoresis after 40 cycles (Fig. 6B). The expression level of ABHD1 was, on average, about 7% that of Sec12. However, in tests the expression of ABHD1 was greater than Sec12. In the steady-state situation in tissues, there was a positive correlation (r= 0.73) between the expression of ABHD1 and Sec12 mRNA relative to the expression of β-actin and not an inverse correlation, which would have suggested that RNA interference occurred.

ABHD1 and Sec12 mRNAs were expressed in all cultured cell types examined, being found in smooth muscle, fibroblasts, endothelial, epithelial and blood cell types (Fig. 7A and 7B). The expression of ABHD1 in fibroblasts was notably low relative to other cell types. On average, the expression level of ABHD1 was 1.4% that of Sec12. In the cell types, there was no correlation between the expression of ABHD1 and Sec12 mRNA.
The human
Discussion

The human ABHD1 and Sec12 genes contain a short conserved overlapping region in their 3'UTR, and share 3 base pairs (ATT/AAT) in their polyadenylation signal sequences, a novel feature that has not been identified previously in the human genome. This feature has been found also in two other mammals. The homologues of human ABHD1 in the human genome. This feature has been found also in two other mammals. The homologues of human ABHD1 and mouse Sec12 do not overlap and are not linked in the genomes of the fish, fly, Drosophila melanogaster; mosquito, Anopheles gambiae and the nematode, Caenorhabditis elegans. A single 3' RACE PCR ampiclon was seen after agarose gel electrophoresis for both the human and mouse ABHD1 and Sec12 gene transcripts, indicating that a single polyadenylation signal only is utilised and alternative splicing in both genes is absent. The Sec12/PREB gene transcript was suggested previously to display alternative splicing since most tissues yielded two bands of 2.2 and 1.5 kb on northern blots [29]. However, the lower band is probably due to hybridisation of the Sec12/PREB cDNA probe to the 3'UTR of the ABHD1 transcript.

Overlapping genes are often functionally and/or structurally related and may regulate each others expression by a natural antisense mechanism. Although Sec12 and ABHD1 proteins do not share any sequence homology, it is possible that they could be functionally related. In this regard, it is of interest to note that both Sec12 and ABHD1 have a single transmembrane domain. Sec12 is located in the ER and it is possible that this is the location of ABHD1 also.

If both genes in a gene pair are transcribed simultaneously, the complementary regions in their mRNAs could possibly meet and form double-stranded RNA. Any such double-stranded RNA is liable to be mistaken for viral DNA, leading to the destruction of the double-stranded RNA and homologous mRNAs by the cell's antiviral defence mechanism [39]. This may provide an evolutionary pressure to avoid such regions of gene overlap, unless the formation of double-stranded RNA is one mechanism by which gene translation may be regulated. However, when the chromosomal rearrangement in the common ancestor of man and rodents that, perchance, brought the ABHD1 and Sec12 genes together occurred, the rearrangement became "locked-in" since evolutionary pressure has selected to maintain this polyadenylation site. A mutation in this polyadenylation site would be detrimental to survival. Sec12 transcripts were lost. The human ABHD1 protein may have an enzymatic function such as an esterase, lipase or thioesterase. It is evolutionarily conserved, being found in bacteria such as E.coli, suggesting an important housekeeping role for the enzyme. It is expressed in a wide range of tissues and cells, although its expression level varied greatly. However, it is one of three related genes in...
the genome, being most closely related to ABHD3. It remains to be determined whether ABHD2 and ABHD3 could take over the cellular role of ABHD1 if the ABHD1 gene was lost.

Although both ABHD1 and Sec12 mRNAs were cloned from human lung cDNA, they were expressed in all adult tissues and cell types examined. The expression level of ABHD1 was generally 7% that of Sec12. ABHD1 levels were highest in skeletal muscle, testis and liver, but over a thousand-fold lower in spleen and fibroblasts. A similar tissue expression pattern for ABHD1 has been found in mice [25]. The reasons for the wide variation in expression are not known. However, the expression of ABHD1 was greater than Sec12 in testis, which suggests that ABHD1 may have an important role in this organ. The level of expression of Sec12 was more constant between tissues and cell types, varying less than a hundred-fold.

Figure 5
Chromosomal localisation and structures of the ABHD1 and Sec12 genes. (A) The human ABHD1 and Sec12 genes are at locus 2p23.3 on opposite strands. Genes encoded on + strand are shown above the line. They are flanked by the elastin microfibril interface located protein, EMILIN; ketohexokinase or fructokinase, KHK; cell growth regulatory with EF-hand domain, CGR11; solute carrier family 5 (sodium-dependent vitamin transporter) member 6 in the telomeric direction and by the NAS hypothetical protein, LOC165086; transcription factor 23, TCF23; SLC5A6; apoptosis related protein, APR-3 and the trifunctional protein of pyrimidine biosynthesis, carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase, CAD genes in the centromeric direction. Genes orientated in the opposite direction to ABHD1 are shown below the line. (B) The gene structures of the ABHD1 and Sec12 genes. The human genomic sequence corresponding to these two cDNAs is located on BAC clone RP11-195B17 and both genes have nine exons and CpG islands. The ORF is indicated by closed boxes. The sequences around the initiation methionine codons are shown, as are the stop codons and the polyadenylation sites. The size of the exons and introns are indicated.
The expression of steady-state \textit{ABHD1} and \textit{Sec12} mRNA in both tissues and cells showed that these two genes are co-expressed in a spatial manner. However, their expression may be separated in a temporal manner. There was no evidence of an inverse correlation between the respective levels of both RNA species that would be expected if RNA interference were occurring. However, antisense transcripts may not affect mRNA stability, but may interfere with translation [21]. It remains to be determined whether the formation of double-stranded RNA occurs and if there is an inverse correlation in \textit{ABHD1} and \textit{Sec12} protein levels.

**Conclusions**

The human \textit{ABHD1} cDNA encodes a deduced protein of 405 residues predicted to contain an amino-terminus transmembrane domain and a carboxy-terminus alpha/beta hydrolase fold. The \textit{ABHD1} gene maps to chromosome 2p23.3. \textit{ABHD1} has characteristics of a housekeeping gene, possessing a 5' CpG island and being expressed in all tissues and cells examined. The \textit{ABHD1} and the \textit{Sec12} cDNAs overlap in a tail-to-tail manner in their 3'UTR, and share overlapping polyadenylation signal sequences. There was no inverse correlation between the respective levels of both RNA species to indicate that RNA interference had occurred.

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**Figure 6**

Expression of \textit{ABHD1} and \textit{Sec12} mRNAs in human adult tissues by semiquantitative real time PCR. (A) Expression levels in each tissue cDNA were normalised to the expression levels of the housekeeping gene \(\beta\)-actin. The ratios of \textit{ABHD1} and \textit{Sec12} mRNA/\(\beta\)-actin mRNA (Y axis, arbitrary units) from each tissue were standardised to that of \textit{Sec12} expression in skeletal muscle, which was taken as 100. (B) Ethidium bromide stained agarose gel of PCR products after 40 cycles of amplification. The tissues examined were: heart, He; whole brain, Br; placenta, Pl; lung, Lu; liver, Li; skeletal muscle, SM; kidney, Ki; pancreas, Pa; spleen, Sp; thymus, Th; prostate, Pr; testis, Te; ovary, Ov; small intestine, Sl; colon, Co; peripheral blood leukocyte, Le; and no cDNA control, -c; 100 bp ladder, m.
Methods

Molecular cloning of human ABHD1 and human and mouse Sec12 cDNAs

The mouse ABHD1 cDNA sequence [25] was used in a BLAST search of the human genome sequence to identify the location of the human ABHD1 gene. The genomic BAC clone RP11-195B17 was identified as the likely location of the gene. From the sequence of the BAC clone a PCR primer, AGGAGCCCTGAGGGTCCGAAGCCCC (Amersham-pharmacia Biotech, UK), located at the 5' end of the cDNA, was designed and used to clone the human ABHD1 cDNA by 3' RACE from RACE ready lung cDNA according to the manufacturer's instructions (Clontech, UK). To investigate the nature of the complementary cDNA sequences of human ABHD1 with Sec12, 3' RACE analysis was used to clone the human and mouse Sec12 cDNA sequences and determine the location of the polyadenylation sites. Human and mouse Sec12 electronic contiguous sequences were generated from EST sequences homologous to the rat Sec12/PreB cDNA sequence [29].

PCR primers, GATGAGAGGGTACGGAGTGTCCCG and CAGAGTCCGCTGAGGGGTCCGC were designed from the 5' end of the Sec12 electronic contiguous sequences of human and mouse respectively. Utilising

Figure 7

Expression of ABHD1 and Sec12 mRNAs in human cell types by semiquantitative real time PCR. (A) Expression levels in each cell type cDNA were normalised to the expression levels of the housekeeping gene β-actin. The ratios of ABHD1 and Sec12 mRNA/β-actin mRNA (Y axis, arbitrary units) from each cell type were standardised to that of Sec12 expression in pulmonary adult fibroblasts, which was taken as 100. (B) Ethidium bromide stained agarose gel of PCR products after 40 cycles of amplification. The lanes are: 1, phiX174 DNA/HaelIII markers (m); 2, pulmonary artery smooth muscle (SM); 3, bronchial smooth muscle (SM); 4, HFL-1 (pulmonary foetal fibroblasts) (Fb); 5, pulmonary adult fibroblasts (Fb); 6, placental microvascular endothelial (En); 7, umbilical vein endothelial (En); 8, A549 (adenocarcinoma alveolar epithelial) (Ep); 9, H322 (adenocarcinoma bronchial epithelial) (Ep); 10, HEL (erythroleukemia) (Er); 11, K562 (erythroleukemia) (Er); 12, negative control (-c).
these primers encoding the human and mouse Sec12 cDNA sequences were obtained by 3' RACE from lung cdnas. RACE-PCR was carried out in a PE2400 thermocycler (Applied Biosystems, UK) using Advantage cDNA polymerase (Clontech). PCR products were examined by agarose gel electrophoresis and stained with ethidium bromide. PCR products were cloned and sequenced as previously described [40].

**The expression of the ABHD1, ABHD2 and ABHD3 mRNA in lung tissue**

Total RNA was extracted from lung tissue using guanidine thiocyanate and treated with DNase-I to remove any contaminating genomic DNA (Total RNA isolation system, Promega, UK). Total RNA was reverse transcribed with AMV RNase H-reverse transcriptase (ThermoScript, Life Technologies, UK) using an oligo-dT primer. The cDNA was amplified by PCR with an annealing temperature of 60°C using the PCR primers for: G3PDH, GGAATCT-CCATCACCATTCTCCAGGAGC and GGGCATGCGCT- GAGCTTCCCGTC producing a 486 bp amplicon; ABHD1, CTTGCGCATCTCTTTTGGAGGATAC and CACAGCGG GGAGAAAGGCTCAT producing a 410 bp amplicon; ABHD2 (HSI-2), GATCCGTGCTGATGAAAAGTCTTCTC and CATCTCCCTCAGTGACCTGGGTGA producing a 347 bp amplicon and ABHD3 (AF007152), TTCACGGTTGGGCATGAAAGTCTTCTC and CACAGCGGGAGAAAGGCTCAT producing a 410 bp amplicon; ABHD2 (HSI-2), GATCCGTGCTGATGAAAAGTCTTCTC and CATCTCCCTCAGTGACCTGGGTGA producing a 347 bp amplicon and ABHD3 (AF007152), TTCACGGTTGGGCATGAAAGTCTTCTC and CACAGCGGGAGAAAGGCTCAT producing a 410 bp amplicon. PCR products were examined by agarose gel electrophoresis and stained with ethidium bromide.

**Tissue and cellular distribution of human ABHD1 and Sec12 mRNA by real time PCR**

Human cDNA was analysed for the relative expression of the ABHD1, Sec12 and β-actin mRNA by real time PCR. The 16 adult tissue cdnas (Clontech, UK) were generated from polyA+ selected RNA and reverse transcribed using an oligo-dt primer. The 10 cell type cdnas were generated from total RNA and reverse transcribed using random hexamers. PCR was carried out on a GeneAmp 5700 Sequence Detection System using a SYBR Green I double-stranded DNA binding dye assay (both from AB Applied Biosystems). Approximately 4 ng of cDNA from each tissue, and cdna derived from 50 ng of total RNA from each cell type was amplified by PCR using Taq Gold polymerase. Tissue and cellular master mixes were divided into gene specific mixes with the addition of PCR primers to a final concentration of 200 μM. The primers were: ABHD1, CCAAGATAGATGGCATCAGGAG (exon 8) and CCTGTATGGGAAAGGCCCACAGA (exon 8/9) producing a 87 bp amplicon; Sec12, GATGGGCCCTTCTACCTGAGAAG (exon 8) and CACAGGAACACTCCGCGGT (exon 8/9) producing a 132 bp amplicon and β-actin, GGCCACGGCTCTCCTC and GTGGCCGTACAGCTTTG-GC producing a 208 bp amplicon. The amplification conditions were; a 10 min hot start to activate the polymerase followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. The number of cycles required for the SYBR Green I dye fluorescence to become significantly higher than background fluorescence (termed cycle threshold \(C_T\)) was used as a measure of abundance. An average \(C_T\) value was determined for each sample. A comparative \(C_T\) method was used to determine gene expression. Expression levels in each tissue and cell type cdna sample were normalised to the expression levels of the housekeeping gene β-actin (\(ΔC_T\)). The ratios of ABHD1 and Sec12 mRNA/β-actin mRNA from each tissue were standardised to that of Sec12 expression in skeletal muscle, which was taken as 100% (\(ΔΔC_T\)). For the cell types, the ratios of ABHD1 and Sec12 mRNA/β-actin mRNA were standardised to that of Sec12 expression in pulmonary adult fibroblasts which was taken as 100%. The formula 2^\(-ΔC_T\) was used to calculate relative expression levels assuming a doubling of the DNA template per PCR cycle. Amplification specificity was confirmed by melting curve analysis and agarose gel electrophoresis.

**Bioinformatics**

Sequence database searches were carried out using BLAST 2.0 [41]. Protein multiple sequence alignments were carried out with the aid of the programme CLUSTAL W using the default parameters [42]. Transmembrane domains were predicted using COLEFHL [43]. The prediction of the alpha/beta hydrolase fold domain and its catalytic triad were carried out using 3D-PSSM version 2.6.0 [44] as previously described [25].

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