Sphingolipids are essential membrane components of eukaryotic cells. Their synthesis is initiated with the condensation of L-serine with palmitoyl-CoA, producing 3-ketodihydrosphingosine (KDS), followed by a reduction to dihydrosphingosine by KDS reductase. Until now, only yeast TSC10 has been identified as a KDS reductase gene. Here, we provide evidence that the human FVT-1 (hFVT-1) and mouse FVT-1 (mFVT-1) are functional mammalian KDS reductases. The forced expression of hFVT-1 or mFVT-1 in TSC10-null yeast cells suppressed growth defects, and hFVT-1 overproduced in cultured cells exhibited KDS reductase activity in vitro. Moreover, purified recombinant hFVT-1 protein exhibited NADPH-dependent KDS reductase activity. The identification of the FVT-1 genes enabled us to characterize the mammalian KDS reductase at the molecular level. Northern blot analyses demonstrated that both hFVT-1 and mFVT-1 mRNAs are ubiquitously expressed, suggesting that FVT-1 is a major KDS reductase. We also found the presence of hFVT-1 variants, which were differentially expressed among tissues. Immunofluorescence microscopic analysis revealed that hFVT-1 is localized at the endoplasmic reticulum. Moreover, a proteinase K digestion assay revealed that the large hydrophilic domain of hFVT-1, which contains putative active site residues, faces the cytosol. These results suggest that KDS is converted to dihydrosphingosine in the cytosolic side of the endoplasmic reticulum membrane. Moreover, the topology studies provide insight into the spatial organization of the sphingolipid biosynthetic pathway.

Sphingolipids consist of a hydrophobic ceramide backbone and a hydrophilic polar head group. Mammalian sphingolipids include sphingomyelin, with phosphocholine as its head group, and hundreds of glycosphingolipids with carbohydrate head groups. Biosynthesis of these sphingolipids begins with the condensation of palmitoyl-CoA and L-serine by serine palmitoyltransferase, producing 3-ketodihydrosphingosine (KDS) (3, 4). The carbonyl group at the C-3 position of KDS is then reduced by KDS reductase to generate dihydrosphingosine (DHS) (3–5). Subsequently, DHS is N-acylated by ceramide (dihydroceramide) synthase to form dihydroceramide. A double bond is then formed between the C-4 and C-5 positions of dihydroceramide by dihydroceramide desaturase, creating ceramide (3, 4). This biosynthetic pathway is well conserved, even in lower eukaryotes like the yeast Saccharomyces cerevisiae, with the exception of the final step (4). In the yeast, dihydroceramide desaturase does not exist; hence sphingosine-type ceramide is not produced. Instead, dihydroceramide or phytoceramide serves as the hydrophobic backbone of the sphingolipids, with phytosphingosine (PHS) being generated by hydroxylation at the C-4 position of DHS by C-4 hydroxylase (6, 7).

Most of the enzymes involved in sphingolipid biosynthesis have been identified in recent years, and the contribution of yeast genetic approaches in these studies has been quite significant. LCBI and LCBI2 were identified as genes required for the serine palmitoyltransferase activity in yeast (8–11). Subsequent studies demonstrated that Leb1p and Leb2p are subunits of serine palmitoyltransferase (11). By their homology, mammalian LCBI and LCBI2 were identified and found to possess serine palmitoyltransferase activity (12–14). Similarly, Lag1p and Lac1p share homology and have overlapping functions in dihydroceramide/phytoceramide synthesis in yeast (15, 16). Their homologs, UOG1, TRH1, and TRH4, recently were demonstrated to be involved in dihydroceramide/ceramide synthesis in mammals (17–19). Interestingly, Sur2p, a C-4 hydroxylase found in yeast, shares the same desaturase/hydroxylase superfamily as DES1, which is a mammalian dihydroceramide desaturase (6, 7, 20).

The yeast gene TSC10 was identified in a screening for temperature-sensitive suppressors of the Ca2+-sensitive Δcsg2 mutant (5). This mutant exhibits a defect in the synthesis of mannosylinositol phosphoceramide, one of three myo-inositol-containing sphingolipids in yeast (21). The temperature-
sensitive tsc10 mutants accumulated KDS and lacked KDS reductase activity (5). Moreover, purified recombinant Tsc10p catalyzed the conversion of KDS to DHS in an NADPH-dependent manner, indicating that Tsc10p is a KDS reductase (5).

Although Tsc10p was identified in 1998 (5), experimental evidence identifying its mammalian homolog has remained lacking thus far. In the present study, we demonstrate that the KDS reductase in mammals is encoded by the follicular lymphoma variant translocation-1 (FVT-1) gene, a gene identified previously in follicular lymphoma as being juxtaposed to an immunoglobulin Jκ segment by chromosome translocation (22). With the identity of this mammalian gene and its product in hand, we are now at last able to establish the characteristics for this mammalian KDS reductase and examine its role in sphingolipid synthesis. Northern blotting demonstrated that FVT-1 is expressed throughout the body in all tissues examined, although its expression varied among tissues, suggesting that FVT-1 is a ubiquitous KDS reductase. Although FVT-1 had been predicted to be a secreted protein (22), our immunofluorescence microscopic analysis showed that FVT-1 is localized in the endoplasmic reticulum (ER). Moreover, we also reveal that the domain encompassing its active site is exposed to the cytosol, indicating that sphingolipid synthesis proceeds on the cytosolic leaflet of the ER membrane, at least up to the production of DHS.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Human embryonic kidney (HEK293T) cells and HeLa cells were grown in Dulbecco’s modified Eagle’s medium D6429 or D6046, respectively (Sigma) containing 10% fetal calf serum and supplemented with 100 units/ml penicillin and 100 μg/ml streptomycin. HEK293T cells were grown on collagen-coated dishes. Transfection was performed using LipofectAMINE Plus™ Reagent (Invitrogen).

Construction of tsct10 Yeast Strains—The TSC10 gene was amplified by PCR using yeast genomic DNA and primers TSC10-F (5'-ACTAGTCTGAGCCAGCTGAGC-3') and TSC10-R (5'-CCTGAGCACGGTCCTG-3') and then cloned into pGEM-T Easy (Promega Corp., Madison, WI) to generate pAK558. The Tsc10leu2 construct was generated by replacement of the 0.7-kb BclI-EcoRV region of the TSC10 gene with the LEU2 marker and was used to transform the S. cerevisiae diploid cells KA31 (MATa ura3 his3 leu2/ leu2 his3 leu3 trpl1 trpl1) (23). Transformants were selected on synthetic complete (SC; 0.67% yeast nitrogen base and 2% glucose) plates lacking leucine. One of the clones obtained, designated KHY623, exhibited a Tsc10 gene and the resulting tetrads were dissected onto YPD (1% yeast extract, 2% peptone, and 2% glucose) plates containing 5 μg/ml ampicillin and 20 μg/ml chloramphenicol to early log phase. Isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM) was added to induce expression of MBP-N-hFVT-1. After incubation at 37°C for 2 h, cells were chilled on ice and collected by centrifugation. Cells were suspended in 0.1 M EDTA (pH 8.0). Cells were lysed by sonication in the presence of the protease inhibitor mixture and 1 mM PMSF, and the resulting lysate was cleared by centrifugation at 100,000 g for 5 min at 4°C. The resulting supernatant was further centrifuged at 100,000 g for 30 min at 4°C, and the membrane fraction (pellet) was suspended in buffer B (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10% glycerol, 1 mM dithiothreitol, 1 mM EDTA, 1× protease inhibitor mixture (Complete™, Roche Diagnostics), and 1 mM phenylmethylsulfonyl fluoride (PMSF)) and sonicated. After centrifugation at 300 × g for 3 min at 4°C, the resulting supernatant was used as a total fraction. For preparing soluble and membrane fractions, the total fraction was subjected to centrifugation at 100,000 × g for 1 h at 4°C.

Purification of MBP·ΔN-hFVT-1 Protein—The E. coli cells Rosetta (Novagen (EMD Biosciences, Inc.), La Jolla, CA) bearing the pAK603 plasmid were grown at 37°C in LB (1% tryptone, 0.5% yeast extract, 1% NaCl (pH 7.2)) medium containing 50 μg/ml ampicillin and 20 μg/ml chloramphenicol to early log phase. Isopropyl-β-D-thiogalactopyranoside (IPTG) (1 mM) was added to induce expression of MBP·ΔN-hFVT-1. After incubation at 37°C for 2 h, cells were chilled on ice and collected by centrifugation. Cells were suspended in buffer B (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 20% glycerol, 10 mM 2-mercaptoethanol, 1× protease inhibitor mixture, and 1 mM PMSF) and incubated on ice for 30 min with 1/10 volume of 1 mg/ml lysozyme, suspended in 0.1 M EDTA (pH 8.0). Cells were lysed by sonication in the presence of the protease inhibitor mixture and 1 mM PMSF, and the resulting lysate was cleared by centrifugation at 100,000 × g for 5 min at 4°C. The resulting supernatant was further centrifuged at 100,000 g for 30 min at 4°C, and the membrane fraction (pellet) was suspended in buffer B. After incubation with Triton X-100 (final concentration, 1%) at 4°C for 30 min, solubilized proteins were collected by centrifugation at 100,000 × g for 30 min at 4°C. The resulting supernatant was loaded onto an amyllose resin (New England Biolabs, Inc.), washed with buffer C (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 20% glycerol, 10 mM 2-mercaptoethanol, 1 mM EDTA, and 0.2% Triton X-100), and eluted with buffer C containing 10 mM maltose.

In Vitro KDS Reductase Assay—The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 10% glycerol, 150 mM NaCl, 1 mM EDTA, 10 mM 2-mercaptoethanol, 1× protease inhibitor mixture, and 1 mM PMSF and incubated on ice for 30 min with 1/10 volume of 1 mg/ml lysozyme, suspended in 0.1 M EDTA (pH 8.0). Cells were lysed by sonication in the presence of the protease inhibitor mixture and 1 mM PMSF, and the resulting lysate was cleared by centrifugation at 100,000 × g for 30 min at 4°C. The resulting supernatant was loaded onto an amyllose resin (New England Biolabs, Inc.), washed with buffer C (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 20% glycerol, 10 mM 2-mercaptoethanol, 1 mM EDTA, and 0.2% Triton X-100), and eluted with buffer C containing 10 mM maltose.

Northern Blotting—Poly(A) + RNA blots containing 1 μg of RNA from human tissues (Clontech, BD Biosciences, Palo Alto, CA) and total RNA blots containing 20 μg of RNA from mouse tissues (Seegene Inc., Seoul, Korea) were used. The hFVT-1 fragments were amplified using the following primers:

- GGGAAGAAGTTTACGTTAGAAGACC-3'
- GGATCCATGAAGTTTACGTTAGAAGACC-3'
pAK567 plasmid and primers hFVT1-F1 and hFVT1-R1, whereas the mFVT1-fragments were amplified using pAK565 and primers mFVT1-F1 and mFVT1-R1. These fragments were labeled with [32P]dCTP by random priming using the random primer DNA labeling kit, version 2 (TAKARA Bio Inc., Shiga, Japan), to generate their probes. Hybridization was carried out in ExpressHyb buffer (Clontech, BD Biosciences) for 2 h at 68 °C.

**Immunoblotting and Immunofluorescence Microscopy—** Rabbit polyclonal anti-hFVT1-antiserum was raised against the middle region of the hFVT1-protein (amino acid residues 26–292) following expression of the MBP-fused protein. Immunoblotting was performed as described previously (25). Anti-hFVT1-antiserum (1:1000 dilution), anti-calnexin (H-10) antibody (0.2 μg/ml; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), and a peroxidase-conjugated donkey anti-rabbit IgG F(ab’2) fragment (1:7500 dilution) were used. Immunofluorescence microscopic analysis was done as described previously (26). Anti-hFVT1-antiserum (1:1000 dilution), anti-KDEL antibody (1 μg/ml; Stressgen Biotechnologies, Inc., San Diego, CA), Alexa 488-conjugated anti-rabbit antibody (5 μg/ml; Molecular Probes, Inc., Eugene, OR), and Alexa 594-conjugated anti-mouse antibody (5 μg/ml; Molecular Probes, Inc.) were used.

**Protease K Digestion Assay—** HEK293T cells were transfected with pCE-puro hFVT1 were washed with phosphate-buffered saline twice, suspended in buffer D (20 mM HEPES/NaOH (pH 7.5), 0.25M sucrose, 1 mM EDTA, 1× protease inhibitor mixture, and 1 μM PMSF), and lysed using an electric Potter homogenizer for 10 strokes. After removal of cell debris by centrifugation at 30,000 g for 3 min at 4 °C, the supernatant was subjected to ultra centrifugation at 100,000 × g for 1 h at 4 °C. The resulting pellet was suspended in buffer D (without the protease inhibitor mixture or PMSF) and treated with 0.5 mg/ml proteinase K at 4 °C. The resulting pellet was suspended in buffer D (without protease inhibitor mixture or PMSF) and treated with 0.5 mg/ml proteinase K at 4 °C for 2 h in the presence or absence of 1% Triton X-100. After termination of the digestion with 1 μM PMSF, total proteins were precipitated with 5% trichloroacetic acid on ice for 20 min. Protein precipitates were washed with 5% trichloroacetic acid and with acetone and then suspended in SDS sample buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, and a trace amount of bromphenol blue) containing 1 μM PMSF, separated by SDS-PAGE, and subjected to immunoblotting.

**RESULTS**

**Identification of FVT-1—** To identify a mammalian KDS reductase, the GenBank™ data base was searched using the BLAST program for sequences similar to yeast Tsc10p. Additional searches of the database were performed using the SHADE programs (Institute for Animal Health, Surrey, United Kingdom). The corresponding GenBank™ accession numbers are CAA45197 (hFVT1), AY634684 (mFVT1), and CAA85228 (Tsc10p). The corresponding GenBank™ accession number for hFVT1 is CAA45197, mFVT1 is AY634684, or Tsc10p is CAA85228.

**Fig. 1. Sequence comparison of mammalian and yeast KDS reductases.** An alignment of the amino acid sequences of hFVT1, mFVT1, and Tsc10p was generated using the ClustalV (50) and BOX-HD programs (Institute for Animal Health, Surrey, United Kingdom). The corresponding GenBank™ accession numbers are CAA45197 (hFVT1), AY634684 (mFVT1), and CAA85228 (Tsc10p). Black boxes indicate identical residues, and gray boxes show amino acid similarity. The putative transmembrane segments are indicated by lines. Dotted residues indicate the putative active site motif, Tyr-XX-X-Lys, which is conserved in the SDR family. The predicted Gly-XX-X-Gly-XX-segment functioning in NAD(H) or NADP(H) binding is marked with a dashed line.

**FVT-1 Is a KDS Reductase—** First we examined whether ectopic expression of hFVT1 or mFVT1 in the TSC10-null yeast cells complemented the growth phenotype. Because sphingolipids are essential for cell viability, Δtsc10 cells cannot grow normally, unless the addition of DHS or PHS to the medium bypasses the requirement of *de novo* sphingolipid synthesis (5). KHY625 (Δtsc10) cells bearing a vector plasmid could not grow on SC plates lacking uracil (SC – URA plates) in the absence of PHS but grew once it was added to the medium (Fig. 2A). The introduction of pAK287, encoding N-terminally FLAG-tagged TSC10, allowed KHY625 cells to grow in the absence of PHS (Fig. 2A). Moreover, both pAK572 and pAK574 plasmids, encoding the mFVT1 and hFVT1 genes, respectively, enabled the growth of KHY625 cells (Fig. 2A). These results indicate that hFVT1 and mFVT1 are functional homologs of Tsc10p.

We next investigated the KDS reductase activity of hFVT1-in-vitro. HEK293T cells were transiently transfected with pCE-puro hFVT1 plasmid or a control vector plasmid. Cell lysates were prepared and subjected to immunoblotting using anti-hFVT1 antibody. The hFVT1 protein was detected in the lysates from pCE-puro hFVT1 plasmid-transfected cells as a catalytic mechanism based on the Tyr-XX-X-Lys motif has been proposed (33, 34). Tyr-186 and Lys-190 in hFVT1 correspond to this motif. The same motif is found in mFVT1 and Tsc10p (Fig. 1, dotted residues). SDR members require NAD(H) or NADP(H) as a coenzyme for their enzyme activity. A Gly-XX-X-Gly-X-Gly segment, generally characteristic of the coenzyme-binding fold in dehydrogenases (32, 34, 35), is also present in FVT1 and Tsc10p proteins (Fig. 1, dashed line). A single Lys residue is observed at the −3 position in the C termini of both hFVT1 and mFVT1. Lys is known to be important for ER membrane proteins to be retained at the ER, although typically ER retention signals are KXXX and KXXXX sequences (34). Tsc10p contains a KXXX type sequence at its C terminus (Fig. 1).
because FLAG-tagged terminal truncation did not abrogate the function of hFVT-1 which is not conserved in Tsc10p, allowed the expression of the However, removal of the N-terminal hydrophobic stretch, C was produced (Fig. 3).

A significant decrease in KDS levels was observed, and little DHS from HEK293T cells transfected with the vector plasmid, no fractions (2.5 g/M PHS and incubated for 3 days at 30 °C. A, KHY625 cells bearing pAK80 (vector), pAK287 (FLAG-TSC10), pAK572 (mFVT-1), or pAK574 (hFVT-1) plasmids. B, KHY625 cells harboring pAK600 (FLAG-hFVT-1) or pAK602 (FLAG-ΔN-hFVT-1) plasmids.

36-kDa band, consistent with its predicted molecular mass of 36.2 kDa, whereas it was absent in mock-transfected cells (Fig. 3A).

Cell fractionation by centrifugation at 100,000 × g for 1 h demonstrated that most of the hFVT-1 was localized in the membrane fraction (Fig. 3B). Therefore, we performed an in vitro KDS reductase assay using the membrane fractions. In assays using membrane fractions (2.5 μg of proteins) prepared from HEK293T cells transfected with the vector plasmid, no significant decrease in KDS levels was observed, and little DHS was produced (Fig. 3C, lane 6). In contrast, the membrane fractions (2.5 μg of proteins) from HEK293T cells overproducing hFVT-1 converted all of the KDS to DHS (Fig. 3C, lane 8). This conversion was highly efficient and dose-dependent so that as little as 0.125 μg of protein converted most of the KDS present (Fig. 3D). Thus, the expression of hFVT-1 generated cellular KDS reductase activity.

To exclude the possibility that FVT-1 is not a KDS reductase but an activator, we tried to purify recombinant hFVT-1 protein from E. coli, which does not contain sphingolipids or KDS reductase. The full-length hFVT-1 fused N-terminally to MBP (MBP-hFVT-1) failed to express in E. coli (data not shown). However, removal of the N-terminal hydrophobic stretch, which is not conserved in Tsc10p, allowed the expression of the truncated form of MBP-hFVT1 (MBP-ΔN-hFVT-1). This N-terminal truncation did not abrogate the function of hFVT-1 because FLAG-tagged ΔN-hFVT-1 (FLAG-ΔN-hFVT-1) could enable the growth of Δtsc10 cells in the absence of PHS, similarly to its full-length control (FLAG-hFVT-1) (Fig. 2B). Once expressed in E. coli, MBP-ΔN-hFVT-1 was recovered in the membrane fraction. The crude membrane fraction was solubilized with Triton X-100, and MBP-ΔN-hFVT-1 was purified using an amylose resin. Based on Coomasie staining MBP-ΔN-hFVT-1 was purified to near homogeneity (Fig. 4A). Using the purified protein, we performed an in vitro KDS reductase assay. KDS was unchanged upon incubation with buffer (Fig. 4B, lane 4), whereas inclusion of the MBP-ΔN-hFVT-1 protein efficiently converted the KDS to DHS (Fig. 4B, lane 3). This reaction was completely dependent on NADPH (Fig. 4B, lane 1), as NADH could not substitute for NADPH (Fig. 4B, lane 2). Using different concentrations of KDS or NADPH (Fig. 5) we estimated Kₘ values for the reductase activity of the purified protein to be 3 and 28 μM for KDS and NADPH, respectively. These results indicated that hFVT-1 itself possesses an NADPH-dependent KDS reductase activity.

Tissue Distribution of FVT-1—To investigate tissue-specific expression patterns of mFVT-1 and hFVT-1, we performed high stringency Northern blot analysis using mRNA extracted from different tissues. A predominant 2.2-kb mFVT-1 mRNA was detected in heart, spleen, and skeletal muscle (Fig. 6A). The highest expression was observed in placenta. Lung, kidney, stomach, and small intestine expressed high levels of mFVT-1 mRNA, whereas only low levels of expression were detected in heart, spleen, and skeletal muscle (Fig. 6A). Similarly, hFVT-1 mRNA was expressed ubiquitously in the
tissues examined (Fig. 6B). In contrast to mFVT-1, hFVT-1 mRNA was detected as two bands (2.7 and 2.5 kb) (Fig. 6B). In most tissues expression of the 2.5-kb mRNA was higher than that of the 2.7-kb mRNA (Fig. 6B). However, the 2.7-kb mRNA was the major species in placenta, and nearly equal levels of the two mRNAs were observed in liver (Fig. 6B). Moreover, the expression profile of hFVT-1 was different from that of mFVT-1. The levels of the hFVT-1 mRNAs were the highest in skeletal muscle and heart and lowest in the colon, thymus, and peripheral blood leukocytes (Fig. 6B). A 1.3-kb hFVT-1 transcript, possibly another splice variant, was detected in the heart and skeletal muscle tissues (Fig. 6B).

FVT-1 Is Localized in the ER—To investigate the subcellular localization of hFVT-1, indirect immunofluorescence microscopic analysis was performed using HeLa cells transfected with a pCE-puro hFVT-1 plasmid for 24 h. hFVT-1 was detected as a reticular structure with a pattern similar to that of ER (Fig. 7A). Therefore, we applied double staining of the cells with the anti-FVT-1 antibody and an antibody directed to the ER retention signal of the ER luminal protein KDEL. The staining pattern of hFVT-1 showed a complete overlap with the ER marker KDEL (Fig. 7B), indicating that hFVT-1 is an ER-resident protein.

The Active Site of FVT-1 Faces the Cytosolic Side of the ER—Both FVT-1 proteins contain a large hydrophilic domain between the putative first and second transmembrane-spanning segments. This domain exhibits similarity to domains of other members in the SDR family and contains the putative NADPH binding site and the active site motif Tyr-X-X-X-Lys. Determination of the membrane topology of FVT-1 is important for identifying on which side of the ER membrane DHS is produced. For this purpose, intact organelles were prepared from HEK293T cells transfected with pCE-puro hFVT-1 and were treated with proteinase K. Calnexin is a type I ER membrane protein with an N-terminal large domain that is exposed to the lumen of the ER (36). We chose calnexin as a control to assess the proper orientation of the isolated ER. Protease treatment reportedly results in the production of C-terminally truncated forms of calnexin (calnexin-ΔC) (36). Accordingly, upon treatment with proteinase K, calnexin was converted to calnexin-ΔC, which reacted with an antibody raised against the N-terminal region of calnexin (amino acid residues 1–70) (Fig. 8A, lane 5). This band disappeared after disrupting the ER membrane with Triton X-100 (Fig. 8A, lane 6), indicating that calnexin-ΔC is protected by the ER membrane. On the other
FVT-1 is a 3-Ketodihydrosphingosine Reductase

During the last decade, most of the mammalian genes responsible for sphingolipid biosynthesis have been identified (12–14, 17–20, 37, 38). However, the identification of the gene for KDS reductase, which is involved in the second step of de novo sphingolipid synthesis, has remained a missing link. We searched for a mammalian gene encoding a product similar to Tsc10p and found FVT-1 as a functional mammalian KDS reductase. First, in Δtsc10 yeast cells forced expression of either human or mouse isoforms of FVT-1 restored the growth defect in the absence of PHS (Fig. 2A). Second, expression of hFVT-1 conferred KDS reductase activity to HEK293T cells (Fig. 3C). Third, purified recombinant MBP-ΔN-hFVT-1 exhibited unequivocal KDS reductase activity (Fig. 4B). Together, these results provide strong support for the hypothesis that FVT-1 is a mammalian KDS reductase.

We found that FVT-1 is localized at the ER (Fig. 7), which is consistent with previous results that sphingolipid biosynthesis, up to the point of ceramide formation, takes place in the ER (39).

FVT-1 appears to be ubiquitous and somewhat conserved because the mouse and human genes share sequence homology and, to some extent, expression patterns. Although it is possible that multiple KDS reductases exist in mammalian cells, the ubiquitous expression of both hFVT-1 and mFVT-1 (Fig. 6) suggests that FVT-1 is a major KDS reductase. Interestingly, two predominant FVT-1 mRNA isoforms (2.5 and 2.7 kb) were detected in human tissues. Expression of the 2.5-kb mRNA was higher than that of the 2.7-kb form in most tissues. However, in the liver their levels were nearly equal, whereas in the placenta the 2.7-kb form was the major species. We found that the cDNA in the GenBank™ with accession number AK025120 comprises a full-length hFVT-1 cDNA. This cDNA is composed of 2044 bp in addition to the poly(A) sequence. Using the BLAST program, we compared the sequence of AK025120 with other human expressed sequence tag clones containing hFVT-1 cDNA and found that most of the expressed sequence tag clones, including AI754522, BQ010432, and BQ020886, have a 3’-untranslated region identical to that of AK025120. However, some expressed sequence tag clones, such as BM995782, BQ182543, and CA438131, contain an extra 100 bp in the 3’-untranslated region. Therefore, the two mRNA species detected by Northern blotting (Fig. 6) may reflect these differences in the length of the 3’-untranslated region.

FVT-1 was originally identified in a study searching for genes involved in tumor progression (22). Variant t(2;18)(p11; q21) chromosome translocation, noted in follicular lymphomas, induced the expression of the BCL-2 proto-oncogene, although rearrangement of BCL-2 was not observed by Southern blot analysis (40). Subsequent study revealed that in this translocation, a Jx segment is juxtaposed to the FVT-1 gene, which is localized 10 kb upstream of the BCL-2 locus (22). FVT-1 was weakly expressed in all of the normal hematopoietic tissues tested, whereas high levels of expression were observed in some T-cell malignancies (22). At the present time, any involvement of FVT-1 in tumorigenesis remains unclear.

In the report in which hFVT-1 was first identified, hFVT-1 was predicted to be a secreted protein based on the presence of the N-terminal hydrophilic stretch (22), although its localiza-
FVT-1 Is a 3-Ketodihydrosphingosine Reductase

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FVT-1 Is a Mammalian 3-Ketodihydrosphingosine Reductase with an Active Site That Faces the Cytosolic Side of the Endoplasmic Reticulum Membrane

Akio Kihara and Yasuyuki Igarashi

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