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Genomic characterization of human SEC14L1 splice variants within a 17q25 candidate tumor suppressor gene region and identification of an unrelated embedded expressed sequence tag

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Abstract. Human SEC14L1 shows partial sequence homology to the budding yeast SEC14 protein and the Japanese flying squid retinal-binding protein and was previously generally localized to 17q25. We more precisely mapped SEC14L1 within a discrete region of 17q25 that likely harbors at least one putative breast and ovarian tumor suppressor gene. We determined that this gene consists of 18 exons ranging in size from 70 bp (exon 11) to 3088 bp (exon 17) and spanning at least 58 kb of DNA. Exon 17 contained a highly polymorphic variable number of tandem repeats (VNTR) and was present only in the larger ubiquitously expressed 5.5-kb transcript. The 3.0-kb ubiquitously expressed transcript included sequences at the beginning of exon 17 (designated exon 17a) and the end of exon 17 (designated exon 18), but lacked the internal 2439 bp of exon 17, including the VNTR. This alternative splicing resulted in a predicted protein of 719 residues from the smaller transcript with four more terminal amino acids than the 715 residue protein predicted from the larger transcript. EST H49244 spanned exon 11 of SEC14L1 and was specifically expressed in human peripheral blood leukocytes. One intragenic single nucleotide polymorphism (SNP) was confirmed. SEC14L1 contained the CRAL/TRIO domain also found in alpha-tocopherol transfer protein (TTPA) and cellular retinaldehyde-binding protein (CRALBP). As retinoids have been shown to inhibit the growth of breast cancer cells, loss of the proposed SEC14L1 retinal-binding function may contribute to breast tumorigenesis. As TTPA and CRALBP have been implicated in retinitis pigmentosa (RP), altered SEC14L1 expression may contribute to RP in previously unlinked families. Coding exon-specific PCR primers were designed to aid in future expression and mutational analyses.

SEC14L1 (SEC14-like 1) maps to 17q25 by FISH (Chinen et al. 1996) and exhibits strong sequence conservation to the *Saccharomyces cerevisiae* budding yeast SEC14 phosphatidylinositol/phosphatidylcholine transfer protein, a key component in the transport of secretory proteins from the Golgi apparatus (Bankaitis et al. 1989), and to the Japanese flying squid *Todarodes pacificus* retinal binding protein, proposed to be involved in retinoid transport in photoreceptor cells (Ozaki et al. 1994). SEC14L1 hybridizes to variably sized transcripts in adult tissues, suggesting involvement of alternative splicing and/or the presence of additional highly homologous family members (Chinen et al. 1996). Its sequence includes a putative open reading frame of 2145 bp that encodes a predicted 715-amino acid protein and contains a 13-bp VNTR averaging 40–60 units in the 3′ untranslated region. In addition, like its yeast ortholog, the SEC14L1 protein includes the cellular retinaldehyde-binding/triple function domain (CRAL/TRIO; Sha et al. 1998). Members of the SEC14-like protein family include the cellular retinaldehyde-binding protein (CRALBP; Crabb et al. 1988; Intres et al. 1994), the multidomain actin remodeling signal protein Trio (Debant et al. 1996), and the alpha-tocopherol transfer protein (TTPA; Zimmer et al. 2000).

We have more precisely localized SEC14L1 within a genomic contig spanning a candidate breast and ovarian tumor suppressor gene region (Kalikin et al. 1997, 1999). We hypothesize that altered expression of SEC14L1 may contribute to breast tumorigenesis given (i) that proteins modified in the Golgi apparatus are known to play important roles in cell signaling (Hicks et al. 2000; Mancini et al. 2000), (ii) that altered protein trafficking (Carayol et al. 2000) and aberrant glycoprotein glycosylation (Dennis et al. 1999; Meichenin et al. 2000) have been observed in neoplastic cells, and (iii) that SEC14L1 yeast orthologs have recognized roles in Golgi protein transport (Bankaitis et al. 1989; Li et al. 2000). Observations that retinoic acid inhibits the growth of breast cancer cells (Yang et al. 1999) further support our hypothesis that SEC14L1 is a strong positional candidate gene for the 17q25 breast tumor suppressor gene and warrants additional characterization.

Materials and methods

PCR. Total RNA was isolated from human leukocytes and breast cell lines by Trizol extraction (Gibco BRL Life Technologies, Grand Island, NY). cDNA was synthesized by using dT and random hexamer primers with SuperScript II reverse transcriptase (Gibco BRL Life Technologies). Genomic DNA was purified from human leukocytes by standard SDS/proteinase K digestion and phenol/chloroform extraction. Additional DNA samples to assess nucleotide variants were prepared from various tissue samples from anonymous unrelated individuals. BAC DNA was purified through Qiagen-tip 500 columns (Qiagen Inc., Valencia, Calif.) as previously described (Kalikin et al. 2000). PCR was performed with each dNTP at 200 μM, each primer at 0.5 μM, and 1 unit of *Taq* polymerase in Buffer A (Promega Corp., Madison, Wisc.) or using the Expand Long Template PCR System with Buffer 1 as per manufacturer's instructions (Roche Molecular Biochemicals, Indianapolis, Ind.). Table 2 lists primers for exon and nucleotide variant sequencing. PCR products were purified from SeaPlaque low melting temperature agarose (FMC BioProducts, Rockland, Maine) using the QIAquick Gel Extraction Kit (Qiagen Inc.) and cycle sequenced using Thermo Sequenase (USB Corp., Cleveland, Ohio). Primers flanking the VNTR were previously described (Chinen et al. 1996). Extension of EST H49244 sequence was accomplished by RNA ligase-mediated rapid amplification of cDNA ends (RLM-RACE; GeneRacer, Invitrogen Corp., Carlsbad, Calif.) with manufacturer-provided 5′ primers and EST primers 5′-CTCCTGCGGCCCGCACAGACTAAGCAGAC-3′ and nested 5′-ACCGCGTCCAGCTATGGAAGAGGGACACT-3′ and cDNA generated from mammary gland polyA$^+$ RNA (Clontech Laboratories, Palo Alto, Calif.). H49244 MTN probe was amplified from genomic DNA by using 5′-CCTGGCTCACTGTTGGCATTCC-3′ and 5′-AAACTACCGCGTCCAGCTATGG-3′.

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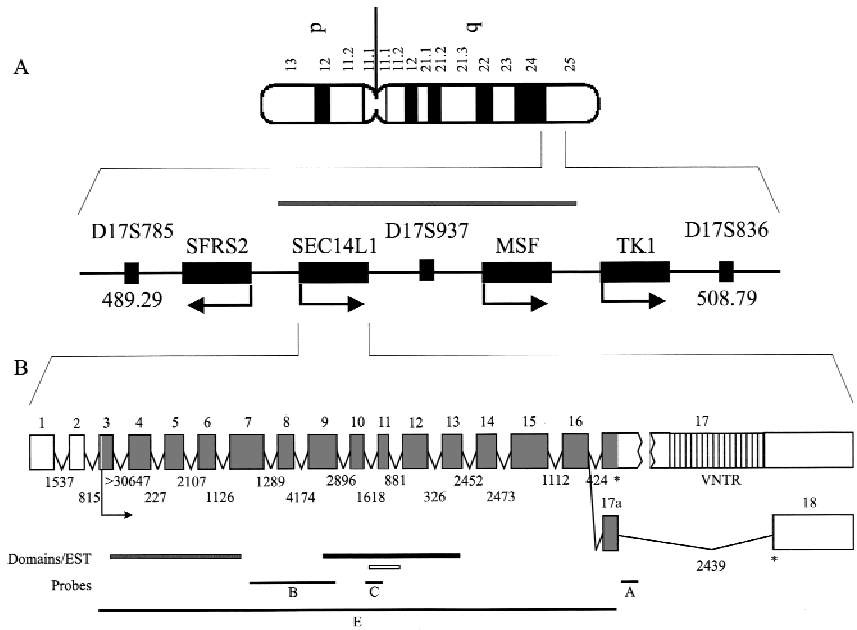

Fig. 1. *SEC14L1* genomic structure. **(A)** Chr 17 ideogram shows location of SEC14L1 and surrounding genes. Suppressor gene candidate region is indicated by thick gray line. Arrows show direction of transcription. For orientation, D17S785 and D17S836 define the bin interval on GeneMap99-GB4 (http://www.ncbi.nlm.nih.gov/genemap/) with map positions (cR3000) listed below. **(B)** Sequence diagram of SEC14L1 exon/intron structure and domains. Numbered boxes represent exons and are drawn to scale except for exon 17. Black boxes are coding regions. Arrow indicates the common start methionine signal between the two transcript variants, and asterisks indicate the distinct stop codons. Connecting lines between the boxes represent introns and are not drawn to scale. Intron sizes (bp) are below the boxes. Hatched area in exon 17 indicates the VNTR. In Domains/EST, black bar exon 3-7 delimits a SEC14L1 protein family conserved domain of unknown function, black bar exon 9-13 delimits the highly conserved retinal-binding CRAL/TRIO domain, and white bar delimits EST H49244. In Probes, black lines indicate SEC14L1 probes A and B and H49244 probe C hybridized in Fig. 2 and SEC14L1 probe E hybridized in Fig. 3.

Sequence assembly. Partially assembled high-throughput genomic sequences from BACs 140d5 (accession no. AC023287), 774d12 (accession no. AC015803), and 1032g1 (accession no. AC012118) were available from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). Additional sequencing was performed by The University of Michigan DNA Sequencing Core. Sequence assembly was performed using phred and phrap programs (Ewing et al. 1998, Ewing and Green 1998). Contigs were viewed and edited using the consed program (Gordon et al. 1998).

Northern analysis. Human multitissue Northern blots (Clontech Laboratories) were hybridized at 60°C in ExpressHyb (Clontech Laboratories). SUM breast cancer cell lines and human papilloma virus (HPV)-immortalized normal breast cell lines were developed at The University of Michigan (http://www.cancer.med.umich.edu/breast_cell/clines/clines.html) and propagated as indicated (http://www.cancer.med.umich.edu/breast_cell/clines/elab/cell_line.htm). HPV lines were grown under the same conditions as SUM149 supplemented with epidermal growth factor (10 μg/ml) and cholera toxin (100 μg/ml). CAL51 was kindly provided by J. Gioanni (Nice, France) and grown as described (Gioanni et al. 1990). Remaining cell lines were maintained as instructed (http://www.atcc.org/). Cell line total RNA was separated on a 1% MOPS/formaldehyde agarose gel, transferred to Hybond-XL (Amersham Pharmacia, Piscataway, NJ) by standard protocol, and hybridized at 60°C in 0.5 M NaH_2PO_4, 7% SDS, 1 mM EDTA buffer (Church and Gilbert 1984). Probes were generated by random priming (Feinberg and Vogelstein 1983) with [α-^{32}P]dCTP. Filters were washed twice in 2× SSC/0.1% SDS, washed once in 0.1× SSC/0.1% SDS and exposed on Kodak X-Omat AR film.

Results and discussion

Resolution of SEC14L1 genomic organization. The genomic structure of SEC14L1 was determined by genomic DNA and cDNA comparative PCR amplification, sequencing, and in silico analysis. Initially, genomic DNA and cDNA isolated from human leukocytes were used as templates to amplify 200- to 300-bp subfragments of SEC14L1. Products were sequenced to identify splice sites when the amplified genomic DNA product was larger than the cDNA product. Application of this strategy proved to be incomplete as many of the SEC14L1 primer combinations did not amplify in genomic DNA, later determined to be owing to the presence of small exons (less than 100 bp) associated with large introns (over 30 kb). Direct BAC and cosmid sequencing, coupled with in silico analysis of available BAC sequences from GenBank, provided evidence for the remaining exon-intron boundaries. The 5.5-kb SEC14L1 transcript consisted of 17 exons ranging in size from 70 bp (exon 11) to 3088 bp (exon 17; see Fig. 1). The highly conserved splice donor GT and splice acceptor AG dinucleotides were evident at all splice junctions (see Table 1; Burset et al. 2000). The proposed start methionine of the predicted 715-residue protein was within exon 3, and the proposed stop codon was within exon 17, which also contained the 13-bp VNTR. Amplification of the VNTR in over 40 anonymous individuals yielded variably sized products most commonly estimated to contain 40–60 repeat units and a single smallest allele estimated to contain less than 20 repeat units similar to original observations (data not shown; Chinen et al. 1996). The SEC14-like protein family defining CRAB/TRIO domain (ProDom Release 2000.1, accession no. PD002025, http://protein.toulouse.inra.fr/prodom.html) spanned exons 9–13. A second domain (ProDom accession no. PD007507) of unknown function was encoded by exons 3–7. Other proteins that harbor both of these domains are found in *C. elegans*, *D. melanogaster*, and humans (Genbank protein accession nos. Q03606, AAF52383, and BAA24850 respectively) but are currently uncharacterized. The genomic sequence spanned at least 58 kb within the putative tumor suppressor region on 17q25 with one gap between exons 3 and 4. Introns ranged in size from 227 bp (intron 5) to over 30 kb (intron 3).

Characterization of a SEC14L1 variant transcript. Initial characterization of SEC14L1 identified both 5.5-kb and 3.5-kb transcripts expressed in all adult tissues tested as well as a 2.4-kb transcript specifically expressed only in skeletal muscle (Chinen et al. 1996). As every exon was significantly smaller than exon 17 (see Fig. 1), we reasoned that the 2-kb difference in size between the two ubiquitously expressed transcripts would most likely occur in this largest exon. Therefore, we designed multiple PCR primers throughout exon 17 to attempt amplification of a smaller than expected product. A product was amplified from human leukocyte cDNA with a primer from exon 16 and a primer located 3′ of the VNTR in the distal end of exon 17 that was significantly smaller than expected based on the 5.5-kb SEC14L1 sequence (GenBank accession no. NM_003003). Sequencing of this product revealed a splice site between nucleotides 2447 and 4886 that excised 2439 bp of exon 17, including the stop codon and the complete VNTR. Therefore, the 3088-bp sequence encoding a single exon in the 5.5-kb transcript instead consisted of two exons, 17a (101 bp) and

Table 1. SEC14L1 exon-intron splice boundaries.

Exon	Acceptor splice site[a]	Donor splice site[a]	Size (bp)
1	5′ UTR...	...tgacaagag**gt**aggcgcgt	167
2	ttttttaac**ag**ctagacttc...	...acaagacag**gt**aggctcat	104
3	tgttttgc**ag**gtgtgagag...	...ATTATGGCT**gt**aagtactt	92
4	aaatttat**ag**GCCTATGAA...	...CTGAAGAAG**gt**aaaggtcg	149
5	ttttttga**ag**ATTGCAGGA...	...TGCTACACC**gt**gagtaatc	131
6	ttatctgc**ag**GTTCACCCT...	...ATTAAAAAA**gt**gagtgtat	128
7	tgttttaa**ag**GGAAAGGAA...	...CCCCTGACG**gt**gggtctgg	234
8	tacattcc**ag**ACAAACTAG...	...AAGGGCAAA**gt**gagtgtca	109
9	ctcttaat**ag**ATTCCAAAA...	...ACGACAAAG**gt**accggatg	189
10	tcctctgc**ag**ATGGGCGGC...	...CTGAGATAC**gt**aagtgcgc	88
11	ttgtatgt**ag**GTTCTCTCC...	...GCCTATCAG**gt**agatgtgg	70
12	caacctgc**ag**CTCATGGAC...	...TGGACGCTG**gt**gggttgag	171
13	ctcctcac**ag**GTTAGTCCG...	...GAGTGCATG**gt**atgtcctg	134
14	cttcttcc**ag**TGCGAAGTG...	...CCACATGAG**gt**acgtcctc	134
15	tctttttc**ag**ATTCTCATT...	...AGCGTGCAG**gt**aaaatcac	251
16	cttgttct**ag**GGTTCCCAT...	...GGATTTCAG**gt**gcggccac	178
17[b]	gccattac**ag**AGGTTCCAT...	...3′UTR	3088
17a	gccattac**ag**AGGTTCCAT...	...GATCTCCAG**gt**agtgccgc	101
18	tgtgtctc**ag**ATGGCGATT...	...3′UTR	548

[a] Lower-case letters represent intronic or non-coding exonic sequence; upper case letters represent coding sequence. The nearly invariant dinucleotide 5′ splice junction "gt" and 3′ splice junction "ag" are indicated in bold.
[b] The 3088 bp exon 17 is found only in the 5.5-kb transcript and contains the stop codon. 2,439 bp of this exon is removed in the 3.0-kb transcript, splicing together exon 17a and exon 18 sequences, adding four additional terminal amino acids, and creating a novel stop codon in exon 18.

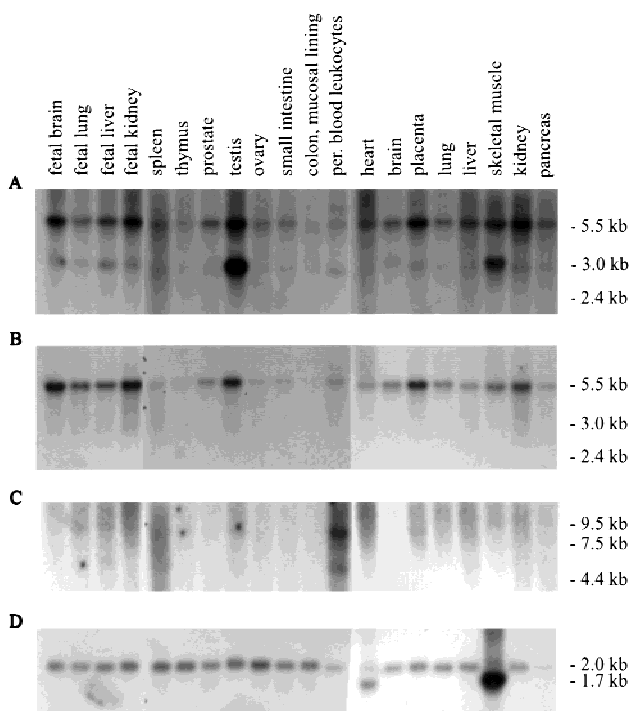

Fig. 2. Confirmation of SEC14L1 genomic structure and EST H49244 expression on human multitissue Northern blots. Location of SEC14L1 probes **A** and **B** and H49244 probe **C** are indicated in Fig. 1. Probe **D** is β-actin to indicate polyA$^+$ RNA loading levels. Bands at 1.7 kb and 2.0 kb in heart and skeletal muscle represent α and γ forms of actin.

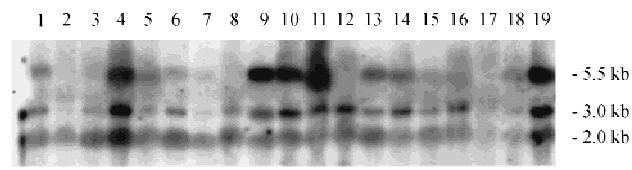

Fig. 3. Northern blot analysis of SEC14L1 in breast cell lines. Probe E (see Fig. 1) hybridizes to 5.5-kb and 3.0-kb transcripts. β-actin probe hybridizes to a 2.0-kb transcript, illustrating total RNA loading level, and is in general agreement with ethidium bromide staining. Cell lines are: 1-BT20, 2-BT474, 3-BT549, 4-CAL51, 5-DU4475, 6-HPV5.12, 7-HPV11.21, 8-HS578T, 9-MCF7, 10-MDA.MB.157, 11-MDA.MB.231, 12-MDA.MB.361, 13-MDA.MD.435, 14-SKBR3, 15-SUM149, 16-SUM159, 17-SUM185, 18-SUM225, and 19-SUM1315.

18 (548 bp), and one intron (2439 bp) in the 3.5-kb transcript (see Fig. 1). These results also predicted the size of the smaller ubiquitously expressed transcript lacking the 2439-bp intron as closer to 3.0 kb than to the previously estimated 3.5 kb (Chinen et al. 1996).

Hybridization of a PCR product spanning the 2439 bp excluded from the 3.0 kb transcript to a human multitissue Northern blot yielded only the expected 5.5-kb transcripts in all adult and fetal tissues tested (see Fig. 1, probe A, and Fig. 2). A coding region RT-PCR product common to both transcripts confirmed expression of both 3.0-kb and 5.5-kb transcripts in all adult tissues tested (Chinen et al. 1996), as well as in all fetal tissues tested (see Fig. 1, probe B, and Fig. 2). However, no 2.4-kb skeletal muscle-specific transcript was observed (Chinen et al. 1996), which may be due to differences in experimental conditions between laboratories. With two exons and one intron in the 3′ end of the 3.0-kb transcript, a protein of 719 residues was predicted that added four amino acids (WRFC) to the terminus of the 715-residue protein predicted from the 5.5-kb transcript before reaching a new stop codon in exon 18. Thus, the presence of two SEC14L1 transcripts different at the 3′ end raises the possibility of variable transcript stability or regulated expression, as 3′ untranslated regions are known to harbor mRNA stability signals (Dean et al. 2001) and localization signals (Derrigo et al. 2000; Mori et al. 2000). In addition, VNTRs have been shown to be biologically important elements in transcription regulation, translational efficiency, mRNA stability, and alteration of protein structure and activity (Nakamura et al. 1998). Indeed, ratios of SEC14L1 3.0-kb and 5.5-kb transcript expression levels varied between some tissues, such as placenta compared with testis (see Fig. 2). Changes in levels of alternatively spliced transcripts also occur between the long and short *C. albicans* SEC14 transcripts during budding and filamentous growth (Riggle et al. 1997). This regulation at the transcript level may then lead to changes in expressed isoform activities, stabilities, and subcellular localizations.

Table 2. SEC14L1 coding exon specific primers.

Exon	Forward primer[a]	Reverse primer[a]	Size (bp)	Annealing (°C)[b]
Exon 3	TAATTTGTTTCTGCATTGTGGT	GCAGATTTTTATTTTCAGAGGC	335	60/58/56
Exon 4	GCAGGACATCAGGTTGTGTC	GATGCCCTCTCCCTCCAG	300	62/60/58
Exon 5	CCCCAGAGACAACATGAACT	CCCTCTAAGACAAAAAGATGCA	320	60/58/56
Exon 6	GCAGGCAGATGATATTCTGTTG	GCATCTTCCACCCAAGAAAT	363	60/58/56
Exon 7	GAAAAATCTTGGGAGAGATGAG	ACTTCCCTAGCCTTGCTCTC	352	60/58/56
Exon 8	AGACTTTGGGAGCCTAAAGC	CGACTTAGATTCCACAGAGACA	335	60/58/56
Exon 9	TCCCTTCACAACTTTTAACATT	GAAGGAGGCCTTGGAAGATA	315	60/58/56
Exon 10	CTCAGTTTCAACTGGGATGG	CTCACTGACGCTTCCACTGT	346	60/58/56
Exon 11	TAGCTGGACGCGGTAGTTTT	CACTAACTGTCTGTCCCTCGTC	302	60/58/56
Exon 12	TTGCTAAGTGTGCTGTCTGTTG	TCCCCTTTCTTAAACAATTTGA	380	60/58/56
Exon 13	GACACTCAGGCAGCAGAGAA	AGCTTAACCCTGGATCAGAGA	344	60/58/56
Exon 14	TGTGTCTGGGTTTCGTGG	AGAGCAGAAAAACGAGTGGA	314	60/58/56
Exon 15	GATCCCCTGGAGAGCACG	GACAGAATCACCTGGCCGT	396	60/58/56
Exon 16	AGGGGTCGGAAGCGAGTC	CCAAGCATTCCTGACACCC	344	62
Exon 17a[c]	GTTGGCAGGGTGGTCCTC	CCGTCCCCTCTGCACACTA	190	54/52/50
Exon 18	TGGTGCTTCCTGTTCCCAAATC	AGGGGATGGCTGCAATTCAAAC	174	58/56/54

[a] Primers are written 5′ to 3′.
[b] When multiple annealing temperatures are listed, reactions are amplified for three cycles at each of the higher temperatures, followed by 27 cycles at the lowest temperature.
[c] Product includes stop codon from the larger 5.5-kb transcript in the flanking 3′ intronic sequences.

Identification of a single nucleotide variant and a novel intronic EST. In sequencing large genomic clones and RT-PCR products from cell lines as well as in silico analysis to assemble the genomic region spanning the SEC14L1 gene, six nucleotide variants within SEC14L1 exonic regions were identified that differed from the published sequence (Genbank accession no. NM_003003; Chinen et al. 1996). Alterations at nucleotides 312 (adenine to guanine), 333 (adenine to guanine), and 981 (thymine to cytosine) occurred at the third position in the nucleotide triplet and did not change the original amino acids. At nucleotide 431 a guanine-to-adenine alteration predicted a change from a serine to a slightly bulkier but still polar asparagine residue. A cytosine-to-thymine change at nucleotide 1030 resulted in an amino acid change from a basic histidine to a polar tyrosine, and a cytosine-to-guanine transversion at nucleotide 1423 changed a polar arginine to a nonpolar glycine. Five of these six nucleotide changes occurred homozygously in samples initially sequenced. Exon-specific PCR products encompassing these variants (exon 3/nucleotides 312 and 333; exon 4/nucleotide 431; exon 8/nucleotide 1030; exon 11/nucleotide 1423) were sequenced from nine additional genomic DNA samples from unrelated individuals and were also all homozygous (data not shown), suggesting that these alterations may represent the true gene sequence. For the sixth change at nucleotide 981, initial sequencing found two samples C/T heterozygous and two other samples C/C homozygous. Sequencing of exon 7 PCR products in 43 additional unrelated individuals identified 19 C/C, 23 C/T, and 4 T/T genotypes (data not shown), further supporting this nucleotide as a SEC14L1 single nucleotide polymorphism (SNP). Comparison of the SEC14L1 protein sequences and these variable residues with the deduced crystal structure of *S. cerevisiae* SEC14 may also aid in determining functional roles of this human ortholog (Sha et al. 1998).

In addition to the nucleotide variants, detailed molecular analysis revealed the presence of EST H49244 (UniGene Cluster Hs.269872; http://www.ncbi.nlm.nih.gov/UniGene/) embedded within SEC14L1. Sequencing of IMAGE clone 1673927 (http://image.llnl.gov/), *in silico* analysis, and 5′ RACE generated 1766 bp of contiguous sequence encompassing H49244, SEC14L1 exon 11, and most of SEC14L1 intronic sequences flanking exon 11. Hybridization to a human multitissue Northern blot with an H49244 PCR product lacking any SEC14L1 sequence showed tissue-specific expression in peripheral blood leukocytes that was clearly distinct from the SEC14L1 expression patterns (see Fig. 2). No open reading frame of substantial length was predicted from this partial sequence, suggesting that it may represent an untranslated region of the transcript. Efforts are ongoing to determine the complete sequence of the gene associated with this novel EST.

Expression of SEC14L1 in breast tumor cell lines. Given the function of the yeast SEC14 ortholog in the Golgi secretory pathway and the presence of the highly conserved CRAL/TRIO domain for binding retinoic acid, an inquiry into the potential role of SEC14L1 in breast tumorigenesis was initiated. Hybridization of an RT-PCR product spanning the coding region of SEC14L1 common to both transcripts to a breast cell line Northern blot consisting of both immortalized normal and tumor samples revealed two transcripts of the expected 3.0-kb and 5.5-kb sizes in all lanes (see Fig. 1, probe E, and Fig. 3). No altered bands were observed. While comparison with the ubiquitously expressed β-actin gene revealed some variability in sample loading between lanes, initial evidence for variable expression ratios between the two transcripts is evident in some samples. For example, MDA.MB.361 (see Fig. 3, lane 12) showed little or no expression of the 5.5-kb SEC14L1 transcript compared with surrounding samples. PCR primers flanking each coding exon were designed and optimized to aid in mutational analysis of these samples as part of the future studies of this gene in breast tumorigenesis (see Table 2). Initial sequence analysis of the SEC14L1 coding region in MDA.MB.361 found no functionally significant nucleotide alterations (data not shown). This suggests that other mechanisms affecting SEC14L1 in this cell line may be involved, including functional changes in control sequences within an untranslated region that affect expression levels or stability. While other differences in transcript expression ratios are apparent, such as between HS578T and MDA.MB.157 (see Fig. 3, lanes 8 and 10 respectively), given that there are no matched normal controls it would be premature to attempt excessive correlation of these data. Therefore, future studies with matched normal/tumor samples will be required.

In summary, we report the characterization of SEC14L1 genomic structure, including 3′ alternatively spliced exons. Translation through these exons produced a second SEC14L1 protein isoform with four additional terminal amino acids. Previously unknown fetal tissue expression was examined and found to be ubiquitous, similar to adult tissue expression. Sequence analysis also revealed one SNP and the presence of EST H49244 within the SEC14L1 sequence. Our initial data in breast tumor cell lines does not clearly implicate a role for SEC14L1 in breast tumorigenesis. However, our breast tumor Northern blot data and the proposed role of SEC14L1 provide initial evidence to warrant further inves-

tigation. In addition, the high homology of SEC14L1 to TTPA and CRALBP provides another possible role for SEC14L1 in disease pathology. Mutations in both TTPA (Yokota et al. 1997) and CRALBP (Maw et al. 1997) have been identified in retinitis pigmentosa (RP) patients, leading to progressive blindness. While TTPA and CRALBP are just two of a number of genes implicated in RP (Phelan and Bok 2000), families exist that are not linked to any of these known genes (Inglehearn et al. 1998) and should, therefore, be investigated for SEC14L1 alterations. Coding exon-specific PCR primers presented here will aid in further investigation of SEC14L1 in these diseases.

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