Selenoprotein T deficiency alters cell adhesion and elevates selenoprotein W expression in murine fibroblast cells

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Abstract

Mammalian selenoproteins have diverse functions, cellular locations, and evolutionary histories, but all use the amino acid selenocysteine (Sec), often present in the enzyme's active site. Only about half of mammalian selenoproteins have been functionally characterized, with most being oxidoreductases. The cellular role of selenoprotein T (SelT), manifesting a CxxU motif in a thioredoxin-like fold and localized to Golgi and the endoplasmic reticulum, is not known. To examine its biological function, we knocked down SelT expression in mouse fibroblast cells and found that SelT deficiency alters cell adhesion and enhances the expression of several oxidoreductase genes, while decreasing the expression of genes involved in cell structure organization, suggesting the involvement of SelT in redox regulation and cell anchorage. Furthermore, we found that the loss of SelT elevates expression of another selenoprotein, selenoprotein W (SepW1). SelT and SepW1 belong to the same protein family, suggesting that SepW1 may functionally compensate for SelT.

Keywords

Introduction

Selenium is a dietary trace element that is essential for human health and is an important component of various metabolic pathways. Many trace elements are used as cofactors of

proteins, but selenium, in addition to serving as a cofactor in some prokaryotic enzymes (Gladyshev et al. 1994), is incorporated into proteins such as selenocysteine (Sec), the 21st naturally-occurring amino acid (Hatfield and Gladyshev 2002). Proteins containing Sec residues are known as selenoproteins, with Sec most often present at the active sites of these proteins (Brown and Arthur 2001). Evolutionary histories, enzymatic activities, tissue expression profiles, and the location of the Sec residue in selenoproteins vary significantly (Kryukov et al. 2003), as reflected in their functional diversity in physiological processes, such as redox regulation (Behne and Kyriakopoulos 2001), thyroid hormone metabolism, and immune response (Beckett and Arthur 2005). Selenium is thought to exert most of its beneficial roles in health through the action of selenoproteins (Hatfield and Gladyshev 2002). However, among the selenoproteins identified, only about half have been functionally characterized, most of which are involved in redox reactions. The biological functions of many others remain unknown.

Selenoprotein T (SelT) is a selenium-containing protein whose cellular function has not been characterized. It was originally identified through in silico studies, cloned and expressed in a mammalian cell line, and confirmed as a selenoprotein (Kryukov et al. 1999). SelT possess a CxxU motif in a thioredoxin-like fold and, based on these characteristics, was assigned to a new protein family, Rdx, which is a thioredoxin-like family (Dikiy et al. 2007). Intracellular localization studies in mouse cell lines suggested that SelT mainly occurs in Golgi and possibly the endoplasmic reticulum (Dikiy et al. 2007); in rat cell lines, it was found to be localized in the endoplasmic reticulum (Grumolato et al. 2008). The latter study suggested a role for SelT in Ca^{2+} homeostasis through a redox mechanism and involvement in neuroendocrine secretion (Grumolato et al. 2008). *Selt* mRNA has also been shown to be elevated in the brains of hypoxia-induced mice, though the reason for this increase is not known (Ikematsu et al. 2007).

To assess the biological function of SelT, its expression was knocked down in mouse fibroblast cells. The resulting data indicate that adhesion ability of stable SelT knockdown cells has decreased. Microarray analyses showed that SelT removal elevated the expression of several oxidoreductase genes, while decreasing the expression of several extracellular matrix genes involved in cell structure organization. The elevated oxidoreductase genes might compensate for the loss of SelT activity, whereas a change in cell adhesion implicates a role in cell anchorage. Interestingly, the loss of SelT increased the expression of selenoprotein W (SepW1), another member of the Rdx family, which might functionally compensate for SelT.

Materials and methods

Materials

Selenium (^{75}Se, specific activity 1000 Ci·mmol^{-1}) in the form of selenious acid was procured from the Research Reactor Facility (University of Missouri, Columbia, Mo.), and neutralized with sodium hydroxide prior to use. Oligonucleotides were procured from Sigma-Genosys (St. Louis, Mo.). Polyacrylamide gels, polyvinylidene fluoride (PVDF) membranes, fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), antibiotic–antimycotic solution, Lipofectamine™ 2000, TRIzol reagent, and Hygromycin B were from Invitrogen (Carlsbad, Calif.); BCA protein assay reagent, SuperSignal West Dura substrate, HRP-conjugated secondary antibodies were from Thermo Fisher Scientific Inc. (Waltham, Mass.); and Cy3 and Cy5 mono-reactive dyes were from GE Healthcare (Chalfont St. Giles, UK). SepW1 antibodies were from Rockland Immunochemicals Inc. (Gilbertsville, Penn.) and β-actin antibodies were from Abcam (Cambridge, Mass.). The Fairplay® II microarray labeling kit was obtained from Stratagene (La Jolla, Calif.) and the CellTiter 96 AQ$_{ueous}$ One Solution cell proliferation assay kit was obtained from Promega

(Madison, Wis.). The iScript™ cDNA synthesis kit and SYBR green supermix were from Bio Rad Laboratories (Hercules, Calif.). The SelT and glutathione peroxidase 4 (Gpx4) antibodies were generated in our laboratories. All other reagents were of the highest grade available.

Generation of Selt siRNA constructs

The pU6-m3 vector (Xu et al. 2007) was used for cloning siSelT targeting sequences and to transfect NIH 3T3 cells as the control. The siRNA Target Finder (Ambion Inc.) was used to select 5 candidate target regions to knockdown the expression of *Selt* (GenBank accession No. NM_001040396), which spanned nucleotides 205–224, 296–316, 562–580, 716–736, and 971–989, respectively. Oligonucleotides containing respective sense and antisense sequences were annealed and cloned into the *Bam*HI–*Hin*dIII restriction sites of pU6-m3. The resulting constructs were designated siSelT-1 through siSelT-5 and verified by sequencing. These constructs and the control plasmid were separately transfected into NIH 3T3 cells and transfected cells were stabilized with 150 μg·mL^{-1} hygromycin B. *Selt* mRNA levels in the knockdown cells were determined by quantitative real-time polymerase chain reaction (qPCR) analysis.

Culture of mammalian cells, transfection, cell growth and cell adhesion assays

NIH 3T3 cells were grown in DMEM supplemented with 10% FBS and antibiotic–antimycotic solution. Transfections with Lipofectamine™ 2000 were carried out according to the manufacturer's protocol. The growth media was supplemented with 100 nmol·L^{-1} sodium selenite and growth rates of cells were assessed by seeding 1×10^5 cells per well in a 6-well tissue culture plate, harvesting after 24, 48, and 72 h with trypsin-EDTA, and counting using the trypan blue extrusion method. The cell adhesion assay was carried out as described (Brake et al. 1990) by seeding 3×10^4 cells onto each well of a 96-well tissue culture plate in triplicate.

Sensitivity of SelT knockdown cells to oxidative stress

The sensitivity of control and SelT knockdown cells to oxidative stress was analyzed by seeding 5×10^3 cells into each well of 96-well plates and growing overnight in DMEM supplemented with 10% FBS. Cells were washed twice with PBS and incubated in serum- and phenol-red-free medium containing hydrogen peroxide for 1 h at 37 °C. Twenty microlitres of CellTiter 96 AQ$_{ueous}$ One Solution reagent was added to each well and incubated for an additional 4 h. The absorbance was recorded at 450 nm and cell viability was expressed as a percentage of untreated controls.

Quantitative real-time PCR

Relative gene expression was verified by qPCR as described (Sengupta et al. 2008), using primer sequences listed in Table 1. Total RNA (1–2 μg) from each sample was reverse transcribed to cDNA using the iScript™ cDNA Synthesis Kit, according to the manufacturer's protocol and used for qPCR in combination with respective primers and SYBR green supermix. Reactions were carried out in triplicate and primer specificity was confirmed by melting curve analysis.

Protein extracts preparation, Western blot analysis and selenoprotein labeling

Protein extracts were prepared from cells using lysis buffer (20 mmol·L^{-1} Tris–HCl, 150 mmol·L^{-1} NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 10 mmol·L^{-1} NaF, 5 mmol·L^{-1} EDTA, and protease inhibitor cocktail) and protein concentration was measured using BCA protein assay reagent. Extracts were electrophoresed on 4%–12% polyacrylamide gels, transferred to PVDF membranes, and immunodetected with antibodies

against SelT (1:800 dilution), β-actin (1:1000 dilution), SepW1 (1:1000 dilution), and Gpx4 (1:1000 dilution). Following incubation with primary antibodies, the membranes were processed as described (Sengupta et al. 2008). To label selenoproteins with ^{75}Se and visualize the labeling, control and SelT knockdown cells were seeded onto a 6 well plate (3 $\times 10^5$ cells per well) and processed as described earlier (Xu et al. 2007).

Microarray analysis

Mouse oligonucleotide glass arrays containing 70mer oligonucleotides were procured from the NCI Microarray Facility (Frederick, Md.). Each slide in these oligonucleotide arrays has 48 blocks containing 28 rows and 28 columns each, with 36960 oligonucleotide spots at a spacing of 155 μm. Probe preparation, hybridization, data processing, and analysis for microarray were done as described by Sengupta et al. (2008). Briefly, total RNA (10–15 μg) isolated from control and SelT knockdown cells was reverse transcribed to cDNA and labeled with monofunctional dye (Cy3 or Cy5) by the Fairplay® II microarray labeling kit (Stratagene) according to the manufacturer's protocol. The Cy3- and Cy5-labeled cDNAs were combined and hybridized to mouse oligonucleotide arrays (designated Mm-MEEBO-v1.3px) then scanned using a Genepix® 4000B scanner and analyzed with GenePix Pro 3.0 software (Axon Instruments). Data were imported into the microarray database (mAdb) and analyzed as described by Sengupta et al. (2008).

Results

Knockdown of SelT in NIH 3T3 cells

Five target regions were selected to knockdown *Selt* mRNA wherein 3 were in the coding region and 2 were in the 3′-untranslated region (3′-UTR) (Fig. 1A). NIH 3T3 cells were stably transfected with the corresponding siSelT constructs and vector. *Selt* mRNA levels were depleted approximately 90% in cells transfected with siSelT-1 or siSelT-5 constructs (Fig. 1B). *Selt* mRNA was depleted 24% in cells transfected with the siSelT-2 construct and remained unchanged in cells transfected with the other constructs (data not shown). As the siSelT-1 and siSelT-5 transfected cells manifested virtually a total loss of *Selt* mRNA, these two cell lines were used for further analysis. Vector-transfected NIH 3T3 cells were designated as control, while cells lacking SelT were designated as SelT knockdown. Control and SelT knockdown cells were labeled with ^{75}Se, protein extracts electrophoresed, and the expression of labeled selenoproteins examined by autoradiography (Fig. 1C). Relative intensities of various ^{75}Se-labeled bands appeared unchanged, whereas SelT was diminished dramatically in the 2 knockdown cell lines (Fig. 1C). The loss of SelT was further confirmed in the knockdown cells by Western blot analysis (Fig. 1D).

Effect of SelT knockdown on cell adhesion, growth, and oxidative stress

Several characteristics of the SelT knockdown and control cells were examined. Both cell lines grew in monolayer, without visible differences in morphology[2] with the growth rate of SelT knockdown cells being slightly slower than in control cells, wherein the reduction was about 17% after 72 h (Fig. 2A). However, upon treatment with trypsin–EDTA, the SelT knockdown cells detached more rapidly from plates as compared with the control cells[2]. To determine if the loss of SelT alters cell adhesion, tissue culture microtiter plates were seeded with 3×10^4 cells for the adhesion assay, as described by Brake et al. (1990). The SelT knockdown cells showed ~40% less adhesion as compared with control cells (Fig. 2B).

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[2]Supplementary data for this article are available on the journal Web site (http://bcb.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 5294. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

Among the functionally characterized selenoproteins, most have been shown to be involved in redox reactions. We therefore examined the sensitivity of the SelT knockdown cells to hydrogen peroxide treatment. There was virtually no difference between SelT knockdown and control cells with respect to their response to exposure to hydrogen peroxide (Fig. 2C).

Effect of SelT knockdown on selenoprotein expression

Metabolic ^{75}Se-labeling of cells showed that the levels of major selenoproteins, Txnrd1, Gpx1, Gpx4, and Sep15, were not affected by the loss of SelT expression (Fig. 1C). The relative expression levels of all mouse selenoprotein transcripts were determined in SelT knockdown and control cell lines by qPCR (Fig. 3A). Transcripts that were below detection limits under these experimental conditions are not shown. The expression of various mRNAs was normalized to the expression of *Gusb* and expression levels of SelT knockdown cells were compared with control cells. The data indicated that although the transcript levels of most selenoproteins analyzed were virtually unchanged, the levels of selenoprotein W (*Sepw1*) showed an increase of about 1.4 fold (Fig. 3A). As the results were averaged for siSelT-1 and siSelT-5 constructs compared with the control, they provide strong evidence that the increase in *Sepw1* is not an effect of off-targeting. These data were corroborated by Western blot analysis wherein the level of SepW1 was found to be higher in SelT knockdown cells compared with control cells (Fig. 3B). The levels of Gpx4 were very similar in the two cells lines, as was the level of β-actin, which was used as a loading control.

Gene expression profile of SelT knockdown NIH 3T3 cells

The gene expression profile of SelT knockdown cells was examined by mouse oligonucleotide arrays containing approximately 29 000 genes. From the arrays, transcripts expressed in at least 50% of the samples and showing an increase or decrease of 2-fold (vs. control) and a *p* value 0.01 were filtered. Following filtering, the genes were grouped according to their functions and sorted as upregulated (Table 2) or downregulated (Table 3) using GENECODIS (Carmona-Saez et al. 2007), DAVID (Dennis et al. 2003), or mAdb software.

Genes elevated in SelT knockdown cells had roles in cell differentiation, cell adhesion, redox regulation, organ development, and protein metabolism (Table 2), whereas those genes with decreased expression were involved in cell structure organization and regulation of transcription (Table 3). Several genes significantly upregulated in SelT knockdown cells such as carbonyl reductase 3 (*Cbr3*), cytochrome P450 family 7 subfamily a polypeptide 1 (*Cyp7a1*), glutathione *S*-transferase mu 1 (*Gstm1*), and superoxide dismutase 1 (*Sod1*), are involved in redox control. The levels of the up-regulated genes were verified by qPCR (Fig. 4A) and it was noted that the expression of *Cbr3* was consistent with the microarray data, whereas *Sod1* showed a 2-fold increase. However, immunodetection of Sod1 did not show a significant change (data not shown). Among the downregulated genes, many localized to the extracellular region and had a role in cell structural integrity. We validated these genes by qPCR and found that although the levels of fibrillin 1 (*Fbn1*) and insulin-like growth factor binding protein 7 (*Igfbp7*) were substantially reduced as noted in the microarray analysis, the other downregulated genes involved in cell structure organization (e.g., *Actr6*, *Rarres2*, *Thbs1*) did not decrease as much as was seen in the microarray analysis (Fig. 4B).

Discussion

To elucidate the function of the selenoprotein SelT, we used RNAi technology to knockdown its expression in NIH 3T3 cells. Two stably transfected cell lines, wherein SelT expression was efficiently knocked down by about 90%, grew in monolayer without any

visible morphological or growth changes as compared with control cells transfected with vector. However, the knockdown cells detached more rapidly from culture plates when treated with trypsin. The cell adhesion assay supports this observation, as the SelT knockdown cells show less adhesion in comparison with their control counterparts under identical conditions. Trypsin detaches cells by breaking their adhesion sites and making them spherical in shape, but a contact between the rounded cells and the culture plate is maintained by some retraction fibres (Revel et al. 1974). The SelT knockdown cells round up and detach faster than control cells, presumably due to the lack of retraction fibres. Interestingly, many downregulated genes observed by microarray analysis in SelT knockdown cells localized to the extracellular region and associated with maintaining cell integrity. Fibrillin 1 (*Fbn1*), a glycoprotein, which is an important constituent of the structural microfibrils (Sakai et al. 1986), has diminished expression in SelT knockdown cells and may be responsible for the decreased adhesion of these cells.

Examination of other selenoprotein transcripts in SelT knockdown cells revealed an increase in the levels of *Sepw1*, which was confirmed by Western blot assays. Though the subcellular and tissue localization profiles of SepW1 and SelT are different, both are members of the Rdx protein family characterized by a thioredoxin-like fold and a conserved CxxU/C motif (Dikiy et al. 2007). The presence of the CxxU/C motif in representatives of the Rdx family suggests a role in redox regulation. SepW1 possesses antioxidant properties (Jeong et al. 2002) and we speculate that its increase in SelT knockdown cells might be a functional compensation by SelT deficient cells. The redox function of SelT is further suggested by the fact that gene expression analysis of SelT knockdown cells by microarray show a highly upregulated level of *Cbr3* gene product. Consistent with the microarray data, a significant 7-fold increase in mRNA expression was observed for *Cbr3* by qPCR analysis. *Cbr3*, a monomeric protein (Watanabe et al. 1998), is a member of the short-chain dehydrogenase/ reductase (SDR) family of proteins, consisting of enzymes with NAD(P)H-dependent oxidoreductase activity (Hoffmann and Maser 2007), but not much is known about the murine protein. Interestingly, loss of thioredoxin reductase in yeast also led to an increase in the levels of *Cbr3* (Bondareva et al. 2007) and reports have indicated that human carbonyl reductases reduce lipid carbonyl groups, generated from lipid peroxidation during oxidative stress to protect cells from oxidative damage (Doorn et al. 2004). The similarity in elevation of *Cbr3* in mammalian cells lacking SelT and yeast lacking thioredoxin reductase might reflect a protective effect against oxidative damage, but such a proposition warrants further investigation.

In summary, the present study shows that loss of SelT in murine fibroblast cells alters adhesion of the resultant cells and treatment with trypsin hastens detachment of these cells from support used in growing the cells. SelT-deficient cells also manifest elevated levels of two genes involved in redox regulation, namely *Cbr3* and *Sepw1*, whose expression levels may functionally compensate for the lack of SelT, supporting the proposal that this protein, like many other selenoproteins, serves an oxidoreductase role. Therefore, the current study extends the functional role of SelT to redox regulation and cell adhesion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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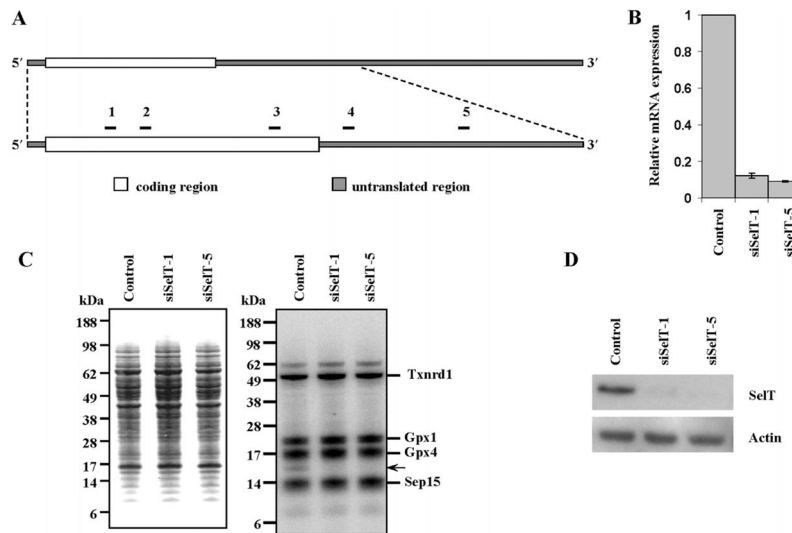
Fig. 1.
Knocking down SelT in murine fibroblast cells. (A) Schematic representation of siSelT target regions (not to scale) for SelT knockdown in NIH 3T3 cells. The position of each selected target site is given in Materials and methods and the two most efficient siSelT sites used in subsequent studies are discussed in the text. (B) The relative *Selt* mRNA levels in NIH 3T3 cells stably transfected with siSelT-1, siSelT-5, or vector and grown in the presence of 100 nmol·L^{-1} sodium selenite, were analyzed by qPCR and normalized to *Gusb*. (C) Protein extracts were prepared from ^{75}Se-labeled cells, electrophoresed, the gels stained with Coomassie blue (left panel), and labeled selenoproteins analyzed by autoradiography (right panel). Selenoproteins identified previously are shown on the right of the ^{75}Se-labeled panel (Kryukov et al. 1999), the band corresponding to SelT is indicated with an arrow, and the corresponding molecular masses of proteins are shown on both panels. (D) Western blot analysis of cell lysates with SelT antibodies, with β-actin as a loading control.
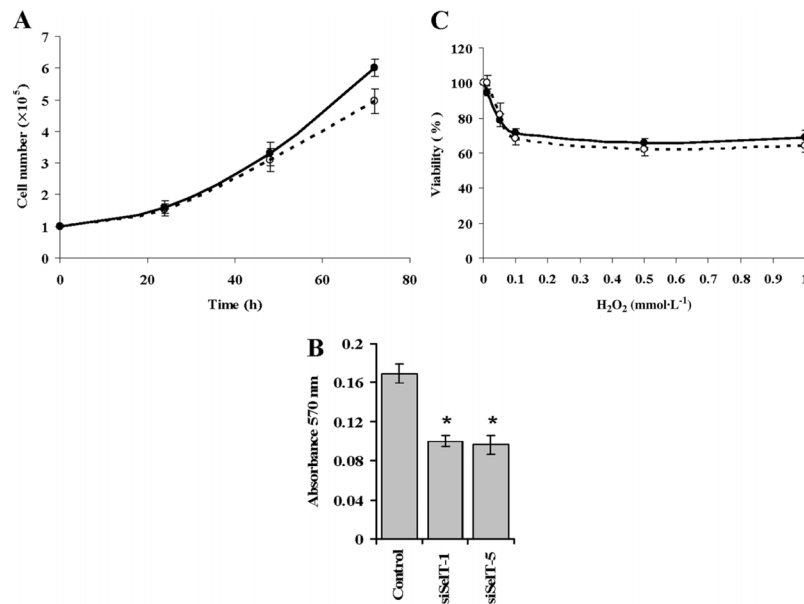
Fig. 2.
Adhesion, growth rate, and sensitivity to oxidative stress of NIH 3T3 cells deficient in SelT. (A) Control and the 2 SelT knockdown cells were seeded at a density of 1×10^5 cells per well in a 6-well culture dish and cell numbers counted at 24, 48, and 72 h intervals to assess growth rates. (B) The adherence of control and SelT knockdown cells plated in a 96-well tissue culture plate was measured by optical density reading (570 nm) as described in Materials and methods. (* p 0.05). (C) Sensitivity of cells to oxidative stress following treatment with hydrogen peroxide was examined as described in Materials and methods. The growth rates and oxidative stress sensitivity of control cells are shown by filled circles and solid lines (—●—), whereas those of SelT knockdown cells are shown by open circles with dashed lines (–○–). The data plotted for the knockdown cells are the average of siSelT-1 and siSelT-5 cells.
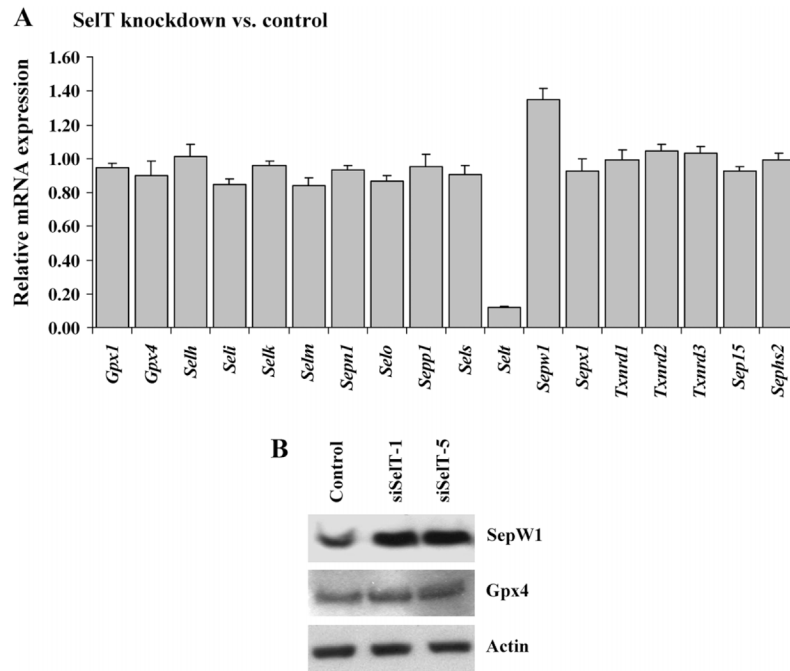
A SelT knockdown vs. control

B

Fig. 3.
Selenoprotein W expression in SelT knockdown cells. (A) Relative expression levels of selenoprotein genes were examined by qPCR analysis. The 2 SelT knockdown lines were compared with control cells and data are means ± SE of the comparison. (B) Protein lysates of SelT knockdown and control cells were analyzed by Western blot assays with SepW1 and Gpx4 antibodies, with β-actin as the loading control.
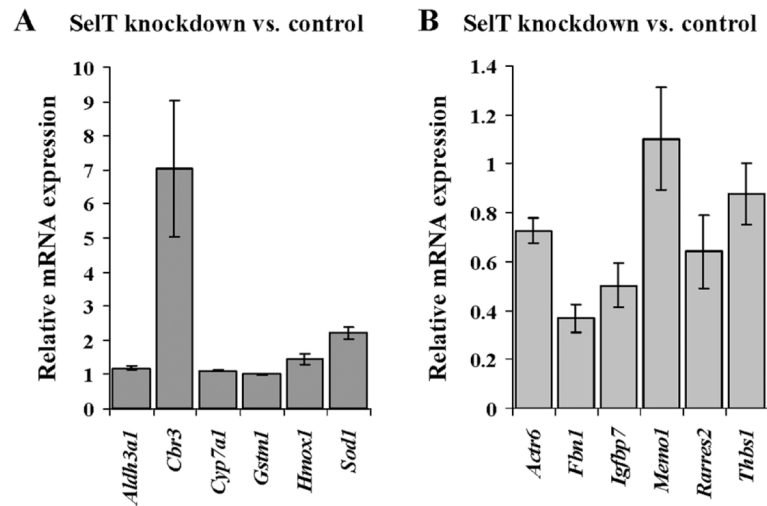
Fig. 4.
Examination of genes altered in SelT knockdown cells. Relative expression levels of genes altered in SelT knockdown cells by microarray in Tables 2 and 3 were analyzed by qPCR. (A) Expression level of genes involved in redox regulation and elevated in SelT knockdown cells. (B) Expression levels of genes decreased in SelT knockdown cells and involved in cell structural organization. Data are a comparison of the 2 SelT knockdown lines and control cells wherein the quantity of RNA for each gene was normalized to *Gusb*. Data shown are means ± SE and the average of 3 analyses for SelT knockdown cells.

Table 1

Primers used in qPCR assays.

Gene	Forward sequence (5′→3′)	Reverse sequence (5′→3′)
Actr6	TTGTCGACAGTGGCTATTCC	GCAGCTGCCTGTAGGATATG
Aldh3a1	GAAGATGCTAAGCAGTCCCA	GGTGGGAGCTATGTATCGTG
Cbr3	GGACAGGATTCTGCTCAATG	CGTGAGGTTCAGTGGCAT
Cyp7a1	TACGACCGGTACCTTGATGA	TGCTTGATTTCTTGGACAGC
Fbn1	AATGAACGGTGGTTGTGAGA	ACTGGCCACCATCACAGATA
Gpx1	CAGGAGAATGGCAAGAATGA	GAAGGTAAAGAGCGGGTGAG
Gpx4	GCAGGAGCCAGGAAGTAATC	GGCTGGACTTTCATCCATTT
Gstm1	CCAAACACACAGGTCAGTCC	CGTCACCCATGGTGTATCTC
Gusb	AACCTCTGGTGGCCTTACCT	CTTGCTCTTTGTGACAGCCA
Hmox1	GCCACCAAGGAGGTACACAT	GCTTGTTGCGCTCTATCTCC
Igfbp7	GCTGGAGAGTATGAGTGCCA	TACAGAGGCTCATGGATTCG
Memo1	ATGTGGAAGACATCCCATTG	CTCCAGCTGCGTAACTGACT
Rarres2	GAGCCTACAGGTGGCTCTG	TTGGTCTGCTGGAGCTTAAA
Selh	CTACCTGTGCAAGTGAACCC	TGAGGCTCAGGAAATTTGAG
Seli	CCGTGTTTGCTCTTCACTTT	GCTGACATGTGATATTGGCA
Selk	TGGTGGATGAGGAAGGTAAA	ACAGAGCAATCCTTGTTTGG
Selm	GATTGGAACCGTCTTCGAG	GTGCTTCATCACCAGGTTGT
Selo	ATCGACTATGGACCCTTTGG	TTTCTGCAGATTCCACTTGC
Sels	CTTTGCGAGGAGGTGGTTAT	CCTTGCTAATGTCAGAGCGA
Selt	TGGTCTAAGCTGGAATCTGG	TTTCGGTGCTGATAGGTAGG
Sep15	TGGAACACAGACAGTGTGGA	TGACCAATGTAAGCATGCAA
Sephs2	GATAGTGCCGTGGTAGGAGA	CTCTGGAAACCACCATCTTG
Sepn1	CCCTAGGTAGCCTTCTGTGC	ACAGGCTGAAGGGTAGCAGT
Sepp1	ATCTTGGCAGCAGTAAGCCT	TCACTTGCTGTGGTGTCTCA
Sepw1	TAGAGGCAGGGTCCTGAAAG	AATCCATCTCTGGCCTGACT
Sepx1	TCCAGTCACTCGAAGTACGC	CTTGCCACAGGACACCTTTA
Sod1	AACCATCCACTTCGAGCAGA	ATGAGGTCCTGCACTGGTACA
Thbs1	CACAAGGGCTCAGGGATACT	GAAATCTTTCCAGCCGATGT
Txnrd1	CTACAGACCATTGCCTTGCT	ACCTCCTACCCACAAGATCC
Txnrd2	TCACTGGAATTGGACTGGAT	ACACAGCCTTTCAGGAACTG
Txnrd3	CTGGAATATGGCTGTTGTGG	AGGTGTTGTTGTCTCTGCCA

Table 2

Genes elevated in SelT knockdown cells.

Symbol	UniGene	Gene description	Fold change	*p* value
Redox regulation				
Aldh3a1	Mm.4257	Aldehyde dehydrogenase family 3, subfamily A1	2.110	0.00002
Cbr3	Mm.4512	Carbonyl reductase 3	6.806	0.00489
Cyp7a1	Mm.57029	Cytochrome P450, family 7, subfamily a, polypeptide 1	2.817	0.00024
Dio2	Mm.21389	Deiodinase, iodothyronine, type II	2.088	0.00024
Gstm1	Mm.37199	Glutathione *S*-transferase, mu 1	2.639	0.00003
Hmox1	Mm.276389	Heme oxygenase (decycling) 1	2.236	0.00004
Sepw1	Mm.42829	Selenoprotein W, muscle 1	2.278	0.00028
Sod1	Mm.276325	Superoxide dismutase 1	2.272	0.00009
Cell differentiation				
Aff4	Mm.395281	AF4/FMR2 family, member 4	2.512	0.00772
Ggnbp1	Mm.440437	Gametogenetin binding protein 1	2.405	0.00015
Igf1	Mm.268521	Insulin-like growth factor 1	2.075	0.00004
Il11ra1	Mm.193451	Interleukin 11 receptor, α chain 1	2.419	0.00090
Runx1t1	Mm.4909	Runt-related transcription factor 1; translocated to, 1	2.224	0.00190
Cell adhesion				
Jup	Mm.299774	Junction plakoglobin	2.460	0.00004
Kif17	Mm.271936	Kinesin family member 17	2.601	0.00010
Rapgef6	Mm.254404	Rap guanine nucleotide exchange factor (GEF) 6	2.476	0.00012
Tctex1d1	Mm.111346	Tctex1 domain containing 1	2.450	0.00058
Smarcd2	Mm.21772	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2	2.380	0.00004
Organ development				
Cul7	Mm.329078	Cullin 7	2.285	0.00128
Foxk1	Mm.24214	Forkhead box K1	2.142	0.00017
Hmgn1	Mm.2756	High mobility group nucleosomal binding domain 1	2.602	0.00001
Osr2	Mm.46336	Odd-skipped related 2	2.351	0.00112
Rpl24	Mm.458082	Ribosomal protein L24	2.807	0.00190
Protein metabolism				
Camk2b	Mm.439733	Calcium/calmodulin-dependent protein kinase II, β	2.405	0.00007
Pigp	Mm.472732	Phosphatidylinositol glycan anchor biosynthesis, class P	2.212	0.00000
Rps2	Mm.328846	Ribosomal protein S2	2.575	0.00573
Rps6kb2	Mm.271937	Ribosomal protein S6 kinase, polypeptide 2	2.414	0.00039
Rps7	Mm.279839	Ribosomal protein S7	2.402	0.00484
Usp37	Mm.66568	Ubiquitin specific peptidase 37	2.923	0.00239

Table 3

Genes decreased in SelT knockdown cells.

Symbol	UniGene	Gene description	Fold change	*p* value
Cell structure organization				
Actr6	Mm.335292	ARP6 actin-related protein 6 homolog	−2.584	0.00054
Fbn1	Mm.271644	Fibrillin 1	−2.770	0.00005
Igfbp7	Mm.233470	Insulin-like growth factor binding protein 7	−2.634	0.00037
Memo1	Mm.311874	Mediator of cell motility 1	−2.866	0.00034
Nupr1	Mm.18742	Nuclear protein 1	−2.164	0.00000
Rarres2	Mm.28231	Retinoic acid receptor responder 2	−2.796	0.00029
Skil	Mm.15406	SKI-like	−2.147	0.00006
Thbs1	Mm.4159	Thrombospondin 1	−2.813	0.00028
Regulation of transcription				
Bptf	Mm.343986	Bromodomain PHD finger transcription factor	−2.478	0.00134
Cnot2	Mm.351553	CCR4-NOT transcription complex, subunit 2	−2.605	0.00081
Mysm1	Mm.208868	Myb-like, SWIRM and MPN domains 1	−2.265	0.00002
Tbp	Mm.244820	TATA box binding protein	−2.965	0.00181