Short Communications

RNAi-mediated knock down of mammalian selenocysteine lyase

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Abstract

Selenocysteine lyase (SCL), which specifically catalyzes the decomposition of L-selenocysteine into Se and L-alanine, is proposed to function as a Se delivery protein to selenophosphate synthetase in selenoprotein biosynthesis. However, the physiological role of SCL has not been established. To address the role of SCL in mammalian cells, we have used RNA-interference (RNAi) to deplete SCL in a HeLa cell line. RNAi-mediated depletion of SCL resulted in growth inhibition in cells cultivated with a serum-containing medium. The similar growth inhibition was observed in SCL-depleted cells cultivated with a serum-free medium supplemented with selenite, selenomethionine, or selenocysteine, indicating that these selenium-containing compounds can not rescue the growth inhibition. These results suggest a physiological importance of SCL in normal cell function. Depletion of SCL may reduce the biosynthesis levels of selenoproteins which are essential for cell proliferation.

Keywords : Selenocysteine, Selenocysteine lyase, Selenoprotein, RNAi, Mammalian cell

Introduction

Selenium (Se) is an essential trace element of many organisms, including humans. Deficiency of Se is related to cancer [1], cardiovascular diseases [2], neural diseases [3], rheumatoid arthritis and immune disorders [4]. Se is incorporated into proteins in the form of selenocysteine residue [5-7]. Selenocysteine lyase (SCL) specifically catalyzes the decomposition of L-selenocysteine into Se and L-alanine and is proposed to function as a selenium delivery protein to selenophosphate synthetase (SPS) in selenoprotein biosynthesis [8-11]. Previously, we reported cDNA cloning and characterization of SCL from mouse liver [9]. The enzyme shows high substrate specificity towards L-selenocysteine and mainly localizes to liver, kidney, testis, spleen, and brain. The distribution pattern of the enzyme is similar to that of SPS, supporting the idea that SCL and SPS may cooperate with each other to function in selenoprotein biosynthesis. We also showed that SCL expression level in stomach was affected by selenite intake. To investigate the cellular role of SCL, we used RNAi to inhibit the expression of the SCL gene. We found that SCL is important for cell proliferation.

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Materials and Methods

Cell Culture and Transfection. Human cervix carcinoma (HeLa) cells used in this study were obtained from the Tissue Culture Shared Resource of the Lombardi Cancer Center (Washington DC). Cells were grown at 37°C under 5% CO_2 atmosphere HeLa cells were cultured in α-minimum essential medium (α-MEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Wako Pure Chemical Industries, Osaka, Japan), 2 mM L-glutamine, and 100 μg/ml penicillin/streptomycin (Biosource International, Camarillo, CA). Sequence of siRNA against the SCL mRNA was 5'-CAAGGCCAGGGACAGACAACA-3' (sense strand), designed by Qiagen (Japan). Transfections were performed with Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. For studies on the effects of selenium depletion, cells (3×10^5 cells/ml) were cultured with α-MEM medium containing 5 μg/ml human insulin, 5 μg/ml human transferrin, 92 nM $FeCl_3$, and 2.5 mg/ml bovine serum

albumin. Selenium was supplemented at 100 nM as L-selenocysteine, L-selenomethionine or sodium selenite.

Cell Viability Assay. For quantification of the degree of cell death in cell culture, we employed the viability assay based on the reduction of tetrazolium compound 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethhoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) and phenazine methosulfate to formazan by mitochondrial dehydrogenase activity. The assay was performed in 96-well microtiter plates. Three wells per sample point were analyzed.

Quantitative Real Time RT-PCR (Q-RT-PCR). Two hundred micrograms of total RNA from HeLa cells were denatured at 70°C for 10 min and then reverse-transcribed in a 30-μl reaction mixture containing 250 μM of each dNTP, 50 units of Superscript II reverse transcriptase (Invitrogen), 80 ng/μl oligo(dT) primers (Invitrogen), 1 × PCR buffer (Applied Biosystems), and 2 mM $MgCl_2$ at 42°C for 50 min. cDNAs were purified on Sepharose G-50 columns (Boehringer Mannheim), dried, and resuspended in 50 μl of dH_2O. Reactions omitting enzyme or RNA were used as negative controls. Q-RT-PCR was performed with LineGene (BioFlux, Tokyo, Japan) using TaqMan One Step RT-PCR Master Mix Reagents (Applied Biosystems, Foster City, CA) and gene-specific primer pairs. For each reaction, standard curves for target and reference genes were made by using six 4-fold serial dilutions of plasmid containing each cDNA sequence. All samples were run in duplicate. Relative amounts of transcripts were calculated by comparison with standard curves. Data were normalized to GAPDH.

Western Blot Analysis. Proteins in cell homogenates were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes. Proteins were detected with an ECL-plus reagent (Amersham Biosciences, Uppsala, Sweden).

Results & Discussion

RNAi-Mediated Silencing of SCL. We first designed siRNAs to target SCL at the SCL-coding sequence. Q-RT-PCR analysis of the SCL mRNA levels in the HeLa cells transfected with the siRNA showed that the siRNA effectively reduced the expression of SCL mRNA by 51% (Fig. 1A). HeLa cells transfected with SCL-siRNA exhibited a marked reduction in the SCL protein level by >60%, as compared with control cells transfected with non-silencing siRNA (Fig. 1B). The

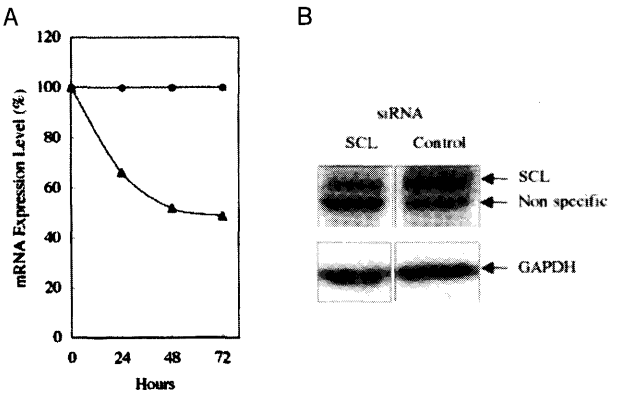

Fig. 1 RNAi-mediated silencing of SCL. (A) Q-RT-PCR analysis of SCL mRNAs. HeLa cells transfected with SCL-siRNA (closed triangle) or control-siRNA (closed circle) were harvested at indicated time, and total RNAs extracted were subjected to Q-RT-PCR analysis. Each mRNA level was normalized to GAPDH. (B) Western blot analysis of HeLa cells transfected with SCL-siRNA or control-siRNA after 72 hours.

effective depletion of SCL in HeLa cells allowed us to study the phenotypical effects associated with SCL deficiency.

Silencing of SCL Results in Cell Growth Defects. We found that HeLa cells transfected with SCL-siRNA did not become confluent and a number of cells floated in the medium and turned spherical in shape, whereas the cells transfected with control siRNA adhered to the culture dish. MTS assay, which detects number of proliferative cells, revealed that silencing of SCL by RNAi severely reduced HeLa cell growth in comparison with the cells transfected with the control siRNA. The growth rate of the SCL-depleted cells was <30% of the control cells (Fig. 2). The growth defects were not due to increased cell death because there were no visible signs of apoptosis (data not shown). Thus, these results suggest that SCL is important for normal cell growth of human HeLa cells. A previous study have shown that selenoproteins are essential to mammalian development or cell proliferation, since removal of the trsp gene coding for $tRNA^{[Ser]Sec}$ in mice is embryonic lethal [12]. In accordance with this, gene knockout of mouse selenoproteins, such as glutathione peroxidase 4 [13], selenoprotein P [14], and mitochondrial thioredoxin reductase [15] is lethal or results in a severe phenotypic abnormality. Therefore, the growth defects of SCL-reduced cells may be caused by the impaired synthesis of one or more essential selenoproteins required for the normal proliferation of mammalian cells.

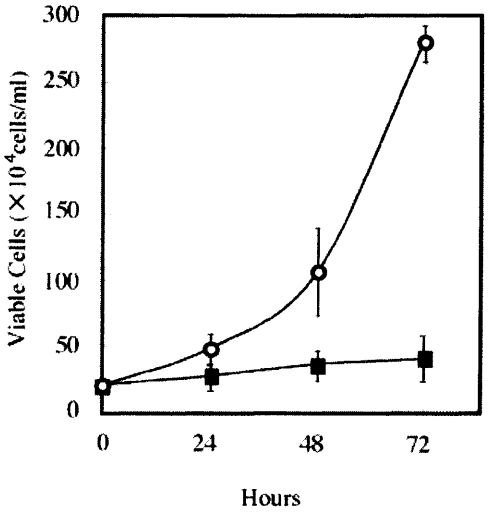

Fig. 2 Growth curves of HeLa cells transfected with SCL-siRNA (closed square) or control-siRNA (open circle). Cells were counted under the microscope.

Cell Growth Defects in SCL-Depleted Cells Was Not Rescued by Selenium Compounds. The essential role of selenium in nutrition has been well established. Without selenium, cells can neither proliferate nor survive. It has been reported that selenium-containing molecules, such as selenomethionine, selenocysteine, selenium-containing proteins in serum, are taken up by cells and can be the cellular source of selenium used for synthesis of selenoprotein [16]. Since SCL provides a selenium substrate for SPS from L-selenocysteine, we examined whether selenium compounds, selenite and selenomethionine, rescue the growth defect of SCL-depleted cells. Growth rate of control siRNA-transfected cells in a serum-free medium supplemented with selenite, selenomethionine, selenocysteine, or serum was similar to one another (Fig. 3A). On the other hand, SCL-depleted cells showed a growth defect phenotype regardless of source of selenium in the medium (Fig. 3B).

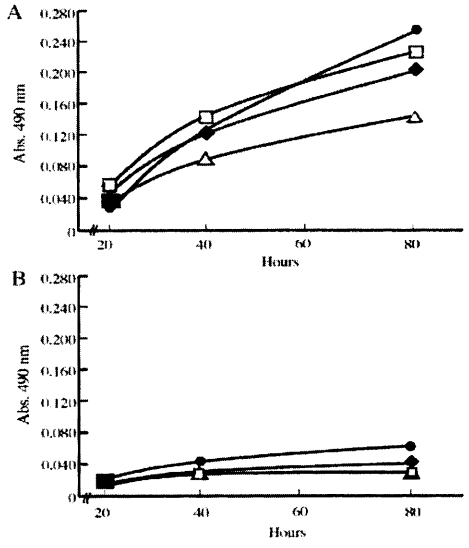

Fig. 3 Growth curves of HeLa cells transfected with control-siRNA (A) or SCL-siRNA (B). Cells were cultivated in a serum-free medium supplemented with serum (closed circle), sodium selenite (open square), L-selenocysteine (closed rhomboid), L-selenomethionine (open triangle).

In cells grown with a serum-containing medium, selenium is mainly supplied as selenium-containing proteins such as selenoprotein P. Selenocysteine can be formed from selenoproteins through proteolysis during protein turnover. Selenomethionine is also converted to selenocysteine via the cysteine biosynthetic pathway [17]. These pathways could provide a source of selenocysteine for the SCL. Our finding that selenite is unable to rescue the growth defect of SCL-depleted cells implies the involvement of SCL in a metabolic pathway which utilizes selenite as a selenium source for selenoprotein biosynthesis.

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