Neoplasia • Vol. 7, No. 11, November 2005, pp. 1030–1038

www.neoplasia.com

SEL1L Affects Human Pancreatic Cancer Cell Cycle and Invasiveness through Modulation of *PTEN* and Genes Related to Cell–Matrix Interactions

Monica Cattaneo, Enrico Fontanella†, Cristina Canton‡, Domenico Delia† and Ida Biunno**

*Institute for Biomedical Technologies (National Research Council), Segrate 20090, Milan, Italy; †Department of Experimental Oncology, Istituto Nazionale Tumori, Milan 20133, Italy; ‡Department of Sciences and Biomedical Technologies, University of Milan, Segrate 20090, Milan, Italy

Abstract

Previously, it was reported that SEL1L is able to decrease the aggressive behavior of human pancreatic tumor cells both *in vitro* and *in vivo*. To gain insights into the involvement of SEL1L in tumor invasion, we performed gene expression analysis on the pancreatic cancer cell line Suit-2 subjected to two complementary strategies: upregulation and downregulation of *SEL1L* expression by stable transfection of the entire cDNA under an inducible promoter and by RNA-mediated interference. SuperArray and real-time analysis revealed that *SEL1L* modulates the expression of the matrix metalloproteinase inhibitors *TIMP1* ($P < .04–.03$) and *TIMP2* ($P < .03–.05$), and the *PTEN* gene ($P < .03–.05$). Gene expression modulations correlate with the decrease in invasive ability ($P < .05$) and in accumulation of *SEL1L*-expressing cells in G1. Taken together, our data indicate that *SEL1L* alters the expression of mediators involved in the remodeling of the extracellular matrix by creating a microenvironment that is unfavorable to invasive growth and by affecting cell cycle progression through promotion of G1 accumulation.

Neoplasia (2005) **7,** 1030–1038

Keywords: *SEL1L*, cell cycle, cancer invasion, extracellular matrix–related genes, siRNA.

Introduction

Most studies on *SEL1L*, the human ortholog of the *Caenorhabditis elegans sel-1* gene [1], have focused on its role in cancer development and have provided significant *in vitro* and *in vivo* evidence to link its increased expression to a decrease in tumor aggressiveness.

Previously, it was reported that ectopic and inducible expression of *SEL1L* reduces the aggressive behavior of breast cancer cells, possibly involving cell–matrix interactions. Moreover, its downregulation has been significantly correlated to poorer outcome in cancer patients [2]. A similar situation was also found in pancreatic cancer, where inducible expression of *SEL1L* in stably transfected cells caused both a decrease in clonogenity and anchorage-independent growth and a delayed tumor growth when inoculated in immunodeficient mice [3]. To investigate the involvement of *SEL1L* in human breast cancer biology, we used proteomic approach and global expression screening (Affymetrix platform) and found that *SEL1L* ectopic expression changed the levels of proteins and transcripts that operate in different signaling pathways and in cytoskeletal reorganization; several tumor-associated proteins were also modulated [4].

SEL1L activation has been reported in the early stages of esophageal, prostate, and non–small cell lung cancers [5,6] (Ferrero et al., unpublished).

The function of SEL1L may be associated with degradation or trafficking of several proteins, as revealed in yeast, plant, and nematode studies. In *Saccharomyces cerevisiae*, the HRD3 protein is required for the degradation of malfolded endoplasmic reticulum (ER)–resident proteins [7]. In *C. elegans*, sel-1, along with ABU-1, is a component of cell survival pathway that is induced when unfolded proteins accumulate in the ER [8]. The SEL1L protein of *Arabidopsis thaliana* is a membrane-anchored glycoprotein that increases under ER stress [9]. It has also been reported that the human *SEL1L* gene is induced in response to ER stress and contributes, along with HRD1, to the protection of cells by degrading unfolded proteins accumulated in the ER [10]. ER stress has been implicated in the pathogenesis of a variety of human diseases, including neural degenerative diseases, diabetes, viral pathogenesis, and cancer [11].

To understand the role of *SEL1L* in human pancreatic cancer, we used the cell line Suit-2 containing the entire *SEL1L* cDNA [3] and two complementary technical strategies: cDNA macroarray (GEArray Q Series Human Tumor Metastasis Gene Array; DBA, Segrate, Italy) and RNA-mediated interference of *SEL1L de novo* transcription.

Address all correspondence to: Ida Biunno, Istituto di Tecnologie Biomediche CNR, *via* F.lli Cervi 93, Segrate 20090, Milan, Italy. E-mail: ida.biunno@itb.cnr.it

Received 8 July 2005; Revised 24 August 2005; Accepted 29 August 2005.

DOI 10.1593/neo.05451

Here we demonstrate that *SEL1L* triggers an antitumor response by modulating the expression of *TIMP1*, *TIMP2*, and *PTEN* genes. All these gene products have widely been studied in several aspects of cancer development and progression [12,13]. Here we also show that gene expression modulations observed in *SEL1L*-expressing cells correlate with their decreased invasive ability and with alteration of cell cycle progression.

Materials and Methods

Cell Culture and In Vitro *Induction of Exogenous* SEL1L *Transcript*

The human pancreatic cancer cell line Suit-2 stably transfected with the entire *SEL1L*-coding region driven by a dexamethasone (DEX)–inducible promoter as well as control mock-transfected cells were grown in RPMI 1640 (Microbiological Associates, Walkersville, MD) supplemented with 10% fetal bovine serum (Hyclone, Euroclone, Devon, UK), penicillin/streptomycin (100 IU/ml), and G418 sulfate antibiotic (200 μg/ml) in a humidified chamber (95% air and 5% CO_2) at 37°C. Cells were regularly seeded to maintain exponential growth.

For experimental purposes, the cells were treated for 7 to 14 days with 1 μM DEX, after which they were harvested and analyzed by reverse transcription–polymerase chain reaction (RT-PCR), Northern blot analysis, and Western blot analysis.

Northern Blot Analysis

Total RNA (20 μg) from cultured cells was isolated using the Total Quick RNA Kit (Talent, Trieste, Italy) and fractionated in denatured agarose gels using well-standardized procedures. Gel-purified, double-stranded DNA probes were labeled with [^{32}P]dCTP using a random primer labeling kit (Promega, Madison, WI). Hybridization was performed overnight at 42°C. RNA loading was normalized by hybridizing the stripped blots with ^{32}P-labeled *HPRT* DNA probe. Northern blots were imaged and quantified using the Quanty one program (Bio-Rad Laboratories S.r.l., Segrate, Italy). The experiments were repeated twice using independent RNA preparations. The oligonucleotide primer sequences used to amplify the DNA probes are as follows:

MMP1	5′-cctccactgctgctgctgct-3′
	5′-gggagagtccaagagaatgg-3′ (770 bp)
MMP7	5′-gaatgttaaactcccgcgtc-3′
	5′-catccgtccagcgttcatc-3′ (390 bp)
TIMP1	5′-ccctggcttctggcatcctg-3′
	5′-ggacctgtggaagtatccgc-3′ (280 bp)
TIMP2	5′-gtagtgatcagggccaaagg-3′
	5′-ctggtacctgtggttcaggc-3′ (320 bp)
PTEN	5′-cgaactggtgtaatgatatg-3′
	5′-catgaacttgtcttcccgtc-3′ (330 bp)
HPRT	5′-aattatggacaggactgaacgtc-3′
	5′-cgtggggtccttttcaccagcaag-3′ (388 bp)

RT-PCR Analysis

Total RNA (1 μg) treated with RNase-free DNaseI (Clontech, Palo Alto, CA) was used in each RT reaction containing 5 μM $MgCl_2$, 1× reaction buffer [50 mM Tris–HCl (pH 8.8), 8 mM $MgCl_2$, 30 mM KCl, and 1 mM dithiothreitol], 1 μM dNTPs, 5 U of RNase inhibitor (RNAsin), 0.8 μg of oligo-$p(dT)_{15}$ primer, 1.6 μg of random primer, and 15 U of Avian Myeloblastosis Virus Reverse Transcriptase (Amersham, Piscataway, NJ). The reaction mixture was incubated for 10 minutes at 25°C, and for 60 minutes at 42°C. The enzyme was denatured at 99°C for 5 minutes and chilled on ice. PCR amplifications were performed with a Perkin-Elmer (Foster City, CA) thermal cycler using 2 μl of RT product per reaction. PCR conditions used to specifically detect *SEL1L* induction and downmodulation were as follows: 94°C for 3 minutes, followed by 20 to 26 cycles at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.

PCR conditions used to detect constitutive *HPRT*, *Activin receptor II*, and *TIMP1–TIMP2–MMP1–MMP7–PTEN–Activin A* expressions were as follows: 3 minutes at 94°C, followed by 23 to 26 cycles at 94°C for 1 minute, annealing at 55°C to 63°C for 1 minute, extension at 72°C for 1 minute, followed by a final extension at 72°C of 5 minutes.

All the PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide.

Sets of primers used to amplify *HPRT* and *MMP7* genes are listed above, whereas the *SEL1L* primer set is as follows:

SEL1L	5′-CCTCAGAGTAATGAGACAGCTCTCC-3′
	5′-GCCACTGGCATGCATCTGAGC-3′ (314 bp)

cDNA Expression Array

The commercially available SuperArray (GEArray Q Series Human Tumor Metastasis Gene Array; DBA) was used to compare the gene expression profiles of the pancreatic cancer cell line stably transfected with *SEL1L* cDNA and pDEX.1 constructs. The arrays were handled following the manufacturer's protocol. Briefly, total RNA (3 μg) from each cell line was reverse-transcribed into ^{32}P-labeled cDNA using MMLV reverse transcriptase. Probes were purified and used to hybridize the filters overnight at 60°C. Blots were then washed twice with 2× SSC/1% SDS and with 0.1× SSC/0.5% SDS at 60°C. Damp membranes were wrapped immediately and exposed to X-ray film. The GEArray Analyzer version 1.2 software was used to interpret the results. The SuperArray contains 96 functionally well-characterized genes involved in several tumor metastatic processes and grouped according to their functions and structural features into seven categories, including growth factors and receptors, cell–cell and cell–matrix interaction molecules, metastasis-associated proteases, protease inhibitors, signal transduction molecules, oncogenes, and metastasis suppressors. Controls are represented by four potentially normalized