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Identification of amyloid β-peptide responsive genes by cDNA microarray technology: Involvement of *RTP801* in amyloid β-peptide toxicity

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Abbreviations: Aβ, Amyloid β-peptide; AD, Alzheimer's disease; CDK, cyclin-dependent kinase; DMEM, Dulbecco's modified Eagle's medium; *DSCR1*, Down syndrome candidate region 1; MTT, 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; RT-PCR, reverse transcription polymerase chain reaction; SDS, sodium dodecylsulfate; *SEST2*, Hi95/sestrin 2; *STC*, stanniocalcin; *ZFP36L2*, zinc finger protein 36.

Abstract

Amyloid β-peptide (Aβ), a causative molecule in the pathogenesis of Alzheimer's disease and the main component of senile plaques, is known to be neurotoxic *in vitro* and *in vivo*. The mechanisms involved in this Aβ-mediated neurotoxicity are not fully understood, although there is evidence to suggest the involvement of oxidative stress, alterations in calcium homeostasis, and/or of CDK activators. Many studies have suggested that Aβ may exert its toxic effect via the activation of transcription factors. Therefore, we investigated Aβ-responsive genes in human neuroblastoma CHP134 cells using 3.1K human DNA microarrays. Among the several genes overexpressed or repressed by Aβ, *RTP801*, Hi95/sestrin 2, and stanniocalcin 2 were confirmed to be Aβ-mediated overexpression in the cells by semiquantitative RT-PCR. Transient expression of the sense *RTP801* gene in CHP134 cells increased sensitivity to Aβ cytotoxicity and the expression of the antisense *RTP801* gene protected the cells from the Aβ toxicity. These results suggest that *RTP801* might play important roles in Aβ toxicity and the pathogenesis of Alzheimer's disease.

Keywords: amyloid β-peptide, cDNA microarray, cytotoxicity, *RTP801*

Introduction

Alzheimer's disease (AD) is one of the major causes of senile dementia. It is a neurodegenerative disorder, which results in the disturbance of learning and memory. Pathologically, insoluble aggregates (amyloid plaques) consisting of amyloid β peptide (Aβ) and cytoskeletal proteins are characteristics of the disease (Mark *et al.,* 1996). The mechanisms involved in the Aβ-mediated neurotoxicity are not fully understood, although a variety of evidence suggests the involvement of oxidative stress (Markesbery, 1997; Miranda *et al.,* 2000; Smith *et al.,* 2000), alterations in calcium homeostasis (Mattson *et al.,* 1998), and/or of CDK activators (Maccioni *et al.,* 2001). Substantial evidence is available to show the involvements of oxidative stress in AD pathogenesis; i) increments of metal ions which accelerate the formation of free radicals (Thompson *et al.,* 1988; Suh *et al.,* 2000), ii) an increase in the oxidation of lipid (Butterfield *et al.,* 1994; Schippling *et al.,* 2000), protein (Smith *et al.,* 1991; Aksenov *et al.,* 1997) and DNA (Lovell *et al.,* 1999), iii) the presence of advanced glycation end products (AGE) (Vitek *et al.,* 1994), malondialdehyde, peroxynitrite, heme oxygenase-1, superoxide dismutase-1 in neurofilament tangles or senile plaques (Pappolla *et al.,* 1992), and iv) production of hydrogen peroxide by β-amyloid peptide (Behl *et al.,* 1994; Huang *et al.,* 1999).

Increments of oxidative stress by Aβ result in the activation of various transcription factors, including NF-κB (Behl *et al.,* 1994; Kaltschmidt *et al.,* 1996) and AP-1 (Marcus *et al.,* 1998; Jang and Surh, 2002). Thus, several studies have been applied to explore Aβ-responsive genes to gain an insight into the molecular mechanisms underlying Aβ toxicity. *Bcl-2*, *Bax* (Paradis et al., 1996), and superoxide dismutase (Aksenov *et al.,* 1998), participated in apoptosis and oxidative stress, were found to be differentially expressed by Aβ. Other studies have been undertaken without the limitations imposed by an *a priori* hypothesis. Gadd45 (Santiard-Baron *et al.,* 1999) and Seladin-1 (Greeve, e*t al.,* 2000) were identified as Aβ-responsive genes by RNA differential displays. Recent advances in cDNA array technology have made it possible to analyze global gene expressions (Park *et al.,* 2002). Calcineurin Aβ was also identified to be upregulated in AD brains by cDNA microarray technology (Hata *et al.,* 2001) and interleukin-8 was found to be overexpressed in Aβ-stimulated postmortem brain microglia (Walker *et al.,* 2001).

In this study, we set out to identify genes differentially expressed in human neuroblastoma CHP134 cells treated with Aβ by using cDNA microarray technology, and we found that *RTP801*, Hi95/sestrin 2 and stanniocalcin 2 (S*TC2*) were overexpressed in Aβ-treated CHP134 cells. Furthermore, the transient expression of *RTP801* in CHP134 cells increased Aβ- or hydrogen peroxide-mediated cytotoxicity.

Materials and Methods

Materials

KNU 3.1K DNA chips were from Tricogene (Daegu, Korea). β-amyloid peptide (1-42) was from the American Peptide Company (Sunnyvale, CA). Dulbecco's modified Eagle's medium (DMEM), Pfx Taq polymerase, Superscript II reverse transcriptase, and a penicillin-streptomycin-fungizone antibiotic solution were from Life technologies, Inc. (Gaithersburg, MD). A pCR3.1 TA cloning kit was from Invitrogen Corp. (Carlsbad, CA), a Ready-to-go T4 DNA ligase mix from Amersham Pharmacia Biotech. (Piscataway, NJ), and gel extraction kits from Qiagen Inc. (Valencia, CA). Primers for Aβ-responsive genes and cloning of *RTP801* and *STC2* were from Biobasic Inc. (Canada) and are listed in Table 1.

Cell culture and Aβ treatment

CHP134 cells were maintained in DMEM containing 10% FBS and antibiotics. Aβ (1-42) was dissolved in sterile DW. For all subsequent experiments, cells were treated with 10 μM Aβ in DMEM +2% FBS for the indicated times.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

CHP134 cells were seeded in 96 well plates (2×10^4 cells/well) in DMEM containing 2% FBS and antibiotics and incubated for 1 day. After discarding the media, cells were treated with Aβ at the indicated concentrations for 48 h. Twenty five μl of MTT solution (5 mg/ml in PBS) was then added into the wells and incubated for 4 h at 37°C. The MTT solution was discarded by aspiration, and the resulting formazan products converted by the viable cells were dissolved in 100 μl of dimethyl sulfoxide. Absorbance at 570 nm was measured using a BioRad M450 microplate reader. Cell survival was expressed as a percentage of Aβ-untreated control cells.

Total RNA and mRNA preparation

Total RNAs were extracted from CHP134 cells treated with or without 10 μM Aβ for 1 h and for 6 h by acid-phenol-guanidinium thiocyanate-chloroform extraction (Chomczynski and Sacchi, 1987). mRNAs were purified using an Oligotex mRNA purification kit (Qiagen Inc.).

Fluorescence-labeled cDNA probe preparation and hybridization

Fluorescence-labeled cDNAs were prepared from mRNAs by RT reaction using the aminoallyl labeling method (http://www.tigr.org/tdb/microarray/protocolsTIGR.shtml). The slides were prehybridized in 0.1% SDS, 5X SSC and 1% bovine serum albumin for 45 min and then hybridized with fluorescence-labeled cDNA probes in 50% formamide, 5X SSC, and 0.1% SDS, 1 μg/μl cot1 DNA, 1 μg/μl poly (A)-DNA for 16-20 h. The slides were then washed in 1X SSC and 0.2% SDS at 42°C for 4 min and in 0.1X SSC and 0.2% SDS at room temperature for 4 min.

Scanning and image analysis

Fluorescence intensities at immobilized targets were measured using Scanarray 4,000 with a laser confocal microscope (GSI Lumonics). The two fluorescent images (Cy3 and Cy5) were scanned separately from a confocal microscope and analyzed using Quantarray software (version 2.0.1, GSI Lumonics). Results were also analyzed by normalizing images to adjust for the different labeling and detection efficiencies at the two different fluorescent wavelengths. We used a filter that included all genes exhibiting a minimum level of expression intensity of more than 1,000 fluorescent units (on a scale of 0-65,535 fluorescent units) for both red and green channels for each experiment.

Reverse transcription-polymerase chain reaction (RT-PCR)

To confirm the differential expressions of genes screen-

Table 1. Primers of Aβ-responsive genes for RT-PCR.

Genes	Sequences	Size (bp)
RTP801	RTP-1056F: CATTGAGTTGTGTGCGGG RTP-1521R: AGGCTTAAACGCAGCTGC	466
	RTP-193F TCACCATGCCTAGCCTTTG RTP-913R CCCCCTCAGGTTGAAGTTC	721
Hi95/Sestrin 2 (SEST2)	SES-744F: CTTAGGTGGCACCATGGC SES-1082R: TTCTGCCTGGAAGCAACC	339
Down syndrome candidate region 1 (*DSCR1*)	DSCR-1401F: TTTGGGATCGGACCTCAG DSCR-2044R: GTCTCTCCCAAACCGGCT	644
Hypothetical protein FLJ20360	FLJ-1604F: CACAGCCCAGGCTGTTCT FLJ-2000R: CCCCACAGGCATACCAAC	397
Stanniocalcin 2 (*STC2*)	STC2-1707F: AAGGGAGTGGCCCCTATG STC2-2105R: GCCAGGACGCAGCTTTAC	399
	STC2-128F: AAGAACCATGTGTGCCGAG STC2-1072R: GGAAAGATTTCGTGGCCA	945
Hypothetical protein MGC4504	MGC-645F: TGGCAGACTTCATGCAGC MGC-1175R: TTCCCAGGGCTATGGATG	531
Zinc finger protein 36, C3H type-like 2 (*ZFP36L2*)	ZFP36-1922F: ACTCGAACTCTGTGCCGG ZFP36-2367R: ACCTATGGGCTGAGGGCT	446
Vaccinia related kinase 1 (*VRK1*)	VRK1-830F: TCCAATGGCTTACTGGCC VRK-1246R: TGGTTCTTGAACGGGTCTG	417
Neuronal PAS domain protein 2 (*NPAS2*)	NPAS2-2280F: ACTTCAGCCATGATCGGC NPAS2-2746R: CTGGAGGCCTGACGACTC	467
Myeloid cell differentiation protein (*MCL1*)	MCL1-1109F: ATATTTTGGGCTTGGGGC MCL1-1433R: CCCTTCCTGGCACAGCTA	325
Coatomer protein complex, epsilon (*COPE*)	COPE-308F: ACTACCTCGCCCACGAGA COPE-828R: GTGCTGGGACAGGACGAT	520
Nucleotide binding protein (*MinD* homolog)	NUBP1-643F: CAACTTCTGCCGCAAGGT NUPB1-1106R: GAAAGTGGCTTCGGACCA	464
Ornithine decarboxylase antizyme 2 (*OAZ2*)	OAZ2-1304F: GTGTGCATTTGCGTCTGG OAZ2-1775R: GGGCAGGCCACTTCTACA	472

ed by DNA chip analysis, RT-PCR was performed (Noh *et al.,* 2001). To synthesize first strand cDNA, one microgram of RNA and 1 μl of 10 μM oligodT (T25NN) were mixed to a final reaction volume of 5 μl and heated for 2 min at 72°C. After cooling on ice for 2 min, RT was performed for 2 h at 42°C in a 10 μl reaction mixture containing 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 6 mM $MgCl_2$, 2 mM DTT, 1 mM dNTP mix and 200 units *MMLV* reverse transcriptase. Following incubation for 10 min at 72°C, the reaction mixture was stored at -70°C. Genes showing differential expression after Aβ treatment were amplified by PCR from the first strand cDNA. All primers used are summarized in Table 1. PCR was performed in a 20 μl reaction volume containing 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, 1 U Taq polymerase, 1 μl first-strand cDNA, and 200 nM primers. The reactions were initial denaturation for 4 min at 95°C, then 30 cycles of; 94°C 15 s, 60°C 15 s, 72°C 1 min; and final extension at 72°C for 10 min. The amplified PCR products were separated in a 1.5% agarose gel containing ethidium bromide and visualized on a UV transilluminator. The levels of amplified DNAs by RT-PCR were quantified using the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas and available from http://ddsdx.uthscsa.edu/dig/itdesc.html) by averaging three separate measurements of each band as well as control.

Cloning of human RTP801 and STC2

Human *RTP801* and *STC2* cDNAs were amplified from total RNAs extracted from CHP134 cells by RT-PCR. The amplified DNA was eluted from the gel using a Qiagen gel extraction kit. The eluted *RTP801* and *STC2* DNAs were then ligated into pCR3.1 vectors using a pCR3.1 TA cloning kit and a Ready-to-go T4 DNA ligase mix. The resulting construct was verified by dideoxy sequencing.

Transient expression of RTP801 and STC2

pCR3.1/RTP801 and pCR3.1/STC2 plasmids were transfected into CHP134 cells using the jetPEI transfection reagent (Qbiogene, Carlsbad, CA) according to the manufacturer's protocols. The transient expressions of *RTP801* and *STC2* were confirmed by semiquantitative RT-PCR.

Statistical analysis

Values are expressed as means±SD. The Student's *t*-test was employed for the analyses. A *P*-value of less than 0.05 was considered statistically significant.

Results

Aβ cytotoxicity in CHP134 cells

Cells were treated with 1, 3 and 10 μM Aβ in media containing 2% FBS for 4 days and cell survival was determined by MTT assay. Cell survival decreased in a dose dependent manner and treatment with 10 μM Aβ resulted in significant cell death ($P < 0.05$) compared to the untreated cells (Figure 1).

cDNA chip analysis

To identify differentially expressed genes in Aβ-treated cells, we used cDNA chips spotted 3,100 human cDNAs derived from the papilla cells of hair follicles. RNA was prepared from CHP134 cells treated with or without 10 μM Aβ for 1 and for 6 h. The initial analysis of the expression data from the cDNA microarrays indicated that the abundance of 13 genes changed 1.5-fold or more during the course of Aβ treatment (Table 2). In order to confirm the induction or repression of the 13 genes, semiquantitative RT-PCR was performed using cDNAs prepared from the RNAs of Aβ-treated or -untreated cells. Six of 13 genes, *RTP801*, stanniocalcin 2 (*STC2*), hypothetical protein MGC4504, Hi95/sestrin 2 (*SEST2*), hypothetical protein FLJ20360, and zinc finger protein 36 (*ZFP36L2*) were confirmed to be Aβ-responsive (Figure 2).

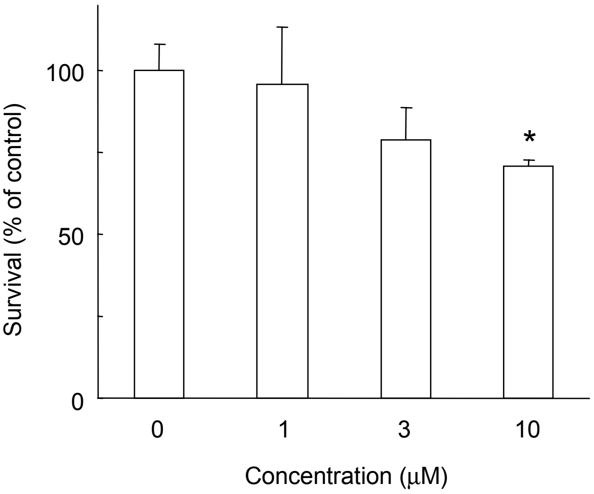

Figure 1. Aβ-induced cell death in CHP134 cells. Cells were treated with the indicated concentrations of Aβ (1-42) and then incubated for 4 days. Cell survival was measured by MTT assay. Values are means ±SD of triplicates of 3 independent experiments. *, $P < 0.05$ (Student's *t*-test).

Induction of *RTP801*, *STC2* and Hi95/SEST2 during Aβ treatment

We further analyzed the expressions of 3 genes, *RTP801*, *Hi95/SEST2*, and *STC2*, with functions known to be associated with the oxidative stress induced by hypoxia (Budanov *et al.,* 2002; Shoshani *et al.,* 2002), and calcium and phosphate homeostasis (Ishibashi *et al.,* 1998), respectively. The levels of these genes were also found to be increased in dose- and time-dependent manners in Aβ-treated cells by RT-PCR (Figure 3).

Effects of *RTP801* and *STC2* on Aβ-mediated cytotoxicity

In an attempt to investigate the effects of *RTP801* and *STC2* on Aβ toxicity, the protein encoding regions of *RTP801* and *STC2* were amplified by RT-PCR and subcloned into a pCR3.1 vector under the control of a cytomegalovirus promoter. The expressions of *RTP801* and *STC2* were increased in the *RTP801*- or *STC2*-transfected cells compared to the vector transfected cells (Figure 4). Transient expression of the sense *RTP801* gene in the cells showed an increase in the Aβ cytotoxicity ($P < 0.01$), and the expression of the antisense *RTP801* gene had a protective effect against Aβ toxicity ($P < 0.05$) compared to the vector-transfected cells (Figure 5). Transient expression of the sense or the antisense *STC2* gene had little effects on Aβ cytotoxicity (Figure 5). These results suggest that *RTP801* might be involved in Aβ cytotoxicity

Table 2. Aβ-induced or repressed genes in CHP134 cells.

Gene name	Folds		Genbank Acc. No.	Function	RT-PCR*
	1 h	6 h			
Stanniocalcin 2 (*STC2*)	0.77	1.85	M_003714	Calcium and phosphate homeostasis	1.41
ypothetical protein MGC4504	0.88	1.79	NM_024111	Unknown	1.36
TP801	0.92	1.71	NM_019058	Cell viability	1.53
Hi95/Sestrin 2 (*SEST2*)	0.79	1.63	NM_031459	Cell viability	1.45
Myeloid cell differentiation protein (*MCL1*)	0.95	0.60	L08246	Apoptosis	1.08
Down syndrome candidate	0.83	0.62	NM_004414	CNS development & transcriptional function	0.90
Ornithine decarboxylase antizyme 2 (*OAZ2*)	0.91	0.66	NM_002537	Regulation of polyamine synthesis	1.07
Coatomer protein complex, epsilon (*COPE*)	1.06	0.60	NM_007263	Vesicle trafficking	0.90
Hypothetical protein FLJ20360	0.72	0.65	NM_017782	Unknown	0.77
Nucleotide binding protein (*MinD homolog*)	0.82	0.61	NM_002484	Nucleotide binding	0.96
Zinc finger protein 36, C3H type-like 2 (*ZFP36L2*)	0.92	0.65	NM_006887	Transcription factor	0.47
Vaccinia related kinase 1 (*VRK1*)	0.95	0.64	NM_003384	Circardian rhythms	0.90
Neuronal PAS domain protein 2 (*NPAS2*)	0.87	0.59	NM_002518	DNA binding	No band

*Values are mean ratios of DNA levels amplified from Aβ (1-42)-treated cells to those from the untreated control.

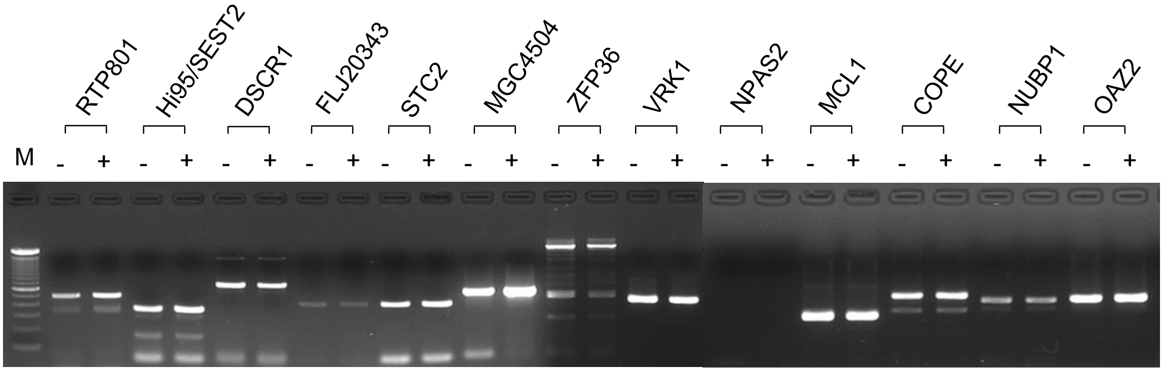

Figure 2. RT-PCR analysis of Aβ-responsive genes. Cells were incubated in the absence (-) or in the presence (+) of 10 μM of Aβ (1-42) for 6 h and harvested. RNAs were purified from the cells and the 1st-strand cDNAs were synthesized with reverse transcriptase. Target sequences for the specific genes were amplified by PCR and the amplified DNAs were analyzed by agarose gel electrophoresis. M, 100 bp ladder. The figure shows representative data from 3 independent experiments.

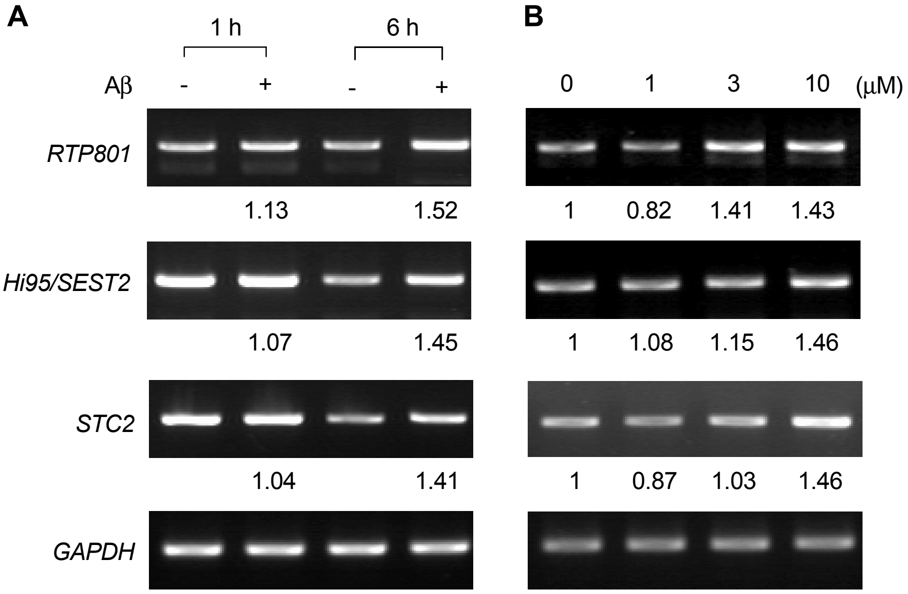

Figure 3. Expressions of RTP801, STC2, and Hi95/SEST2 in CHP134 cells treated with Aβ. A. Cells were incubated in the absence (-) or in the presence (+) of 10 μM of Aβ (1-42) for 1 and for 6 h, and harvested. RTP801 and STC2 were amplified by RT-PCR. B. Cells were treated with the indicating concentrations of Aβ (1-42) for 6 h and harvested. RTP801 and STC2 were amplified by RT-PCR. The amplified DNAs were analyzed by agarose gel electrophoresis and their levels were quantified using a UTHSCSA ImageTool program by averaging three separate measurements of each band as well as control. This shows representative data from 3 independent experiments.

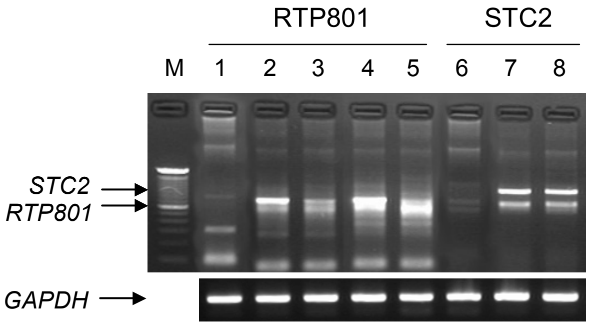

Figure 4. semiquantitative RT-PCR of RTP801 and STC2 in CHP134 cells transfected with pCR3.1/RTP801 or pCR3.1/STC2 vectors. CHP134 cells were transfected with pCR3.1/RTP801 or pCR3.1/STC2 using a jetPEI transfection reagent. After 3 days, cells were harvested and RNAs were extracted. Transient expression of RTP801 and STC2 were confirmed by RT-PCR. RTP-183F and RTP-913R were used in Lane 1 to 5 and STC2-128F and STC2-1072R in Lane 6 to 8. Lanes 1 and 6, vector-transfected cells; Lanes 2 and 3, sense RTP801; Lanes 4 and 5, antisense RTP801; Lanes 7, sense STC2; Lane 8, antisense STC2. M, 100 bp ladder.

and in the pathogenesis of Alzheimer's disease.

Discussion

In the present study we identified six genes as being Aβ-responsive in CHP134 cells by cDNA chip analysis and RT-PCR. *RTP801*, *Hi95/SEST2* and *STC2* were overexpressed in CHP134 cells treated with Aβ and the transient expression of *RTP801* increased their sensitivity to Aβ cytotoxicity. *RTP801* was induced by hypoxia in rat C6 glioma cells regulated by hypoxia-inducible factor-1 (HIF-1) and identified to be involved in apoptosis (Shoshani *et al.,* 2002). Although expression of the *RTP801* gene in MCF7 and PC12 cells inhibited hypoxia- and H_2O_2-mediated apoptosis, its function in cells is not fully understood. *RTP801* is ubiquitously expressed in multiple human tissues at low levels. However, in response to hypoxia its transcription increases rapidly and sharply. The inducible expression of *RTP801* in cells has different biological effects depending on the cell context. Shoshani *et al.* (2002) showed that expression of *RTP801* has protected MCF7 and PC12 cells from hypoxia and from H_2O_2-triggered apoptosis, but detrimentally affected nondividing neuron-like PC12 cells under hypoxia and oxidative stress.

We identified that *Hi95/SEST2* expression was also increased in Aβ-treated CHP134 cells. Hi95/SEST2 has been recently identified as a novel stress-responsive gene involved in the regulation of cell viability (Budanov *et al.,* 2002). *Hi95/SEST2* shares significant homology with a p53-regulated *GADD* family member PA26 (Peeters *et al.,* 2003). Increased expression of Hi95/SEST2 was induced by various cellular stresses

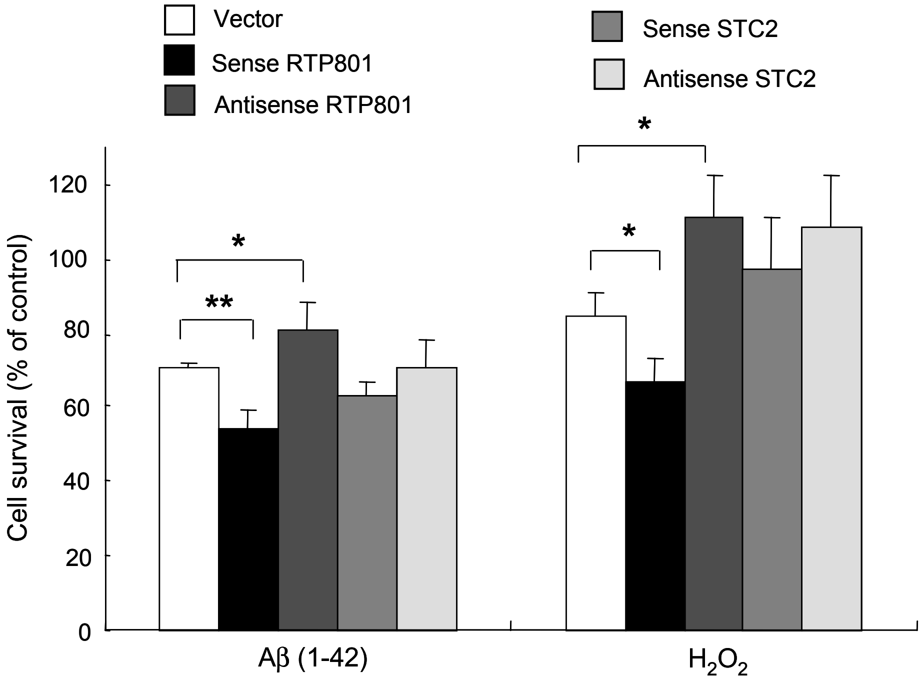

Figure 5. Effects of Aβ and H_2O_2 on cell survival of transient *RTP-801*-or *STC2*-expressed CHP134 cells. CHP134 cells were transfected with a pCR3.1/RTP801 vector. After 4 h, cells were harvested, seeded on 96 well plates (20,000/well), and incubated for 16 h. Cells were treated with 10 μM Aβ (1-42) for 4 days or with 1 mM H_2O_2 for 16 h. Cell survival was measured by MTT assay and compared to the untreated control. Statistical significance was evaluated by the Student's *t*-test (**, $P < 0.01$; *, $P < 0.05$). Values are means±SD of in 3 independent experiments performed in triplicate.

including prolonged hypoxia, oxidative stress, UV-or γ-irradiation, and doxorubicin, and the overexpression of *Hi95/SEST2* full-length cDNA was found to be toxic in many types of cultured cells (Budanov *et al.,* 2002).

Hypoxia is known to induce oxidative stress in PC12 cells via Aβ and reactive oxygen species formation (Green *et al.,* 2002). Aβ also induces oxidative stress itself by producing H_2O_2 (Behl *et al.,* 1994; Huang *et al.,* 1999). Therefore, the Aβ-mediated overexpressions of *RTP801* and *Hi95/SEST2* genes in CHP134 cells might be associated with cellular oxidative stress. Our finding that the transient expression of the sense *RTP801* gene, not the antisense *RTP801* gene, exacerbates Aβ-or H_2O_2-mediated cytotoxicity in CHP134 cells suggests that the overexpression of *RTP801* in Aβ-treated CHP134 cells might play an important role in cell death during Aβ-mediated oxidative stress.

Stanniocalcin (*STC*) is a hormone that was initially identified in fish, which inhibits calcium absorption in the gills and intestines and stimulates the absorption of phosphates (Sundell *et al.,* 1992). Mammalians have two types of *STC*; *STC1* and *STC2*. *STC1* has a 61% homology with fish *STC* and presents in kidney, thyroid glands, ovary, and prostates. *STC2* has a 38% homology with fish *STC2* and presents mainly in the pancreas (Ishibashi *et al.,* 1998). Mammalian *STC1* was suggested to play an important role in calcium homeostasis, including the absorption and secretion of calcium and phosphates. However, the function of *STC2* is unknown (Jellinek *et al.,* 2000). *STC2* might also play an important role in glucose homeostasis (Moore *et al.,* 1999), and was identified as an estrogen-responsive gene, which was induced with estrogen receptor in human breast cancers (Bouras *et al.,* 2002). Our data shows that *STC2* is up-regulated by Aβ treatment. However, the transient expression of sense or antisense *STC2* genes was found to have little effects on Aβ cytotoxicity, suggesting that the Aβ-mediated overexpression of *STC2* may not be directly associated with Aβ toxicity.

In conclusion, our results suggest that some of the novel Aβ-responsive genes play key roles in the response of neuronal cells to Aβ exposure. Further functional analysis of the novel Aβ-responsive genes is required to open up new research routes of enquiry into the pathogenesis of AD.

Acknowledgement

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