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Insights into the Sigma-1 receptor chaperone's cellular functions: a microarray report

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Abstract

We previously demonstrated that Sig-1Rs are critical regulators in neuronal morphogenesis and development via the regulation of oxidative stress and mitochondrial functions. In the present study, we sought to identify pathways and genes that are affected by Sig-1R. Gene expression profiles were examined in rat hippocampal neurons that had been cultured for18 days in vitro (DIV). The cells were transduced with AAV siRNA targeting Sig-1R on DIV 10 for 7 days, followed by gene expression analysis using a rat genome cDNA array. The gene array results indicated that Sig-1R knockdown hampered cellular functions including steroid biogenesis, protein ubiquitination, actin cytoskeleton network, and Nrf-2 mediated oxidative stress. Many of the cellular components important for actin polymerization and synapse plasticity, including F-actin capping protein and neurofilaments, were significantly changed in AAV-siSig-1R neurons. Further, cytochrome c was reduced in AAV-Sig-1R neurons whereas free-radical generating enzymes including cytochrome p450 and cytochrome b-245 were increased. The microarray results also suggest that Sig-1Rs may regulate genes that are involved in the pathogenesis of many CNS diseases including Alzheimer's disease and Parkinson's disease. These data further confirmed that Sig-1Rs play critical roles in the CNS and thus these findings may aid in future development of therapeutic treatments targeting neurodegenerative disorders.

Introduction

Originally proposed as a subtype of the opioid receptor family by Martin and colleagues (Martin et al., 1976), the Sigma-1 receptor (Sig-1R) was later identified as a natural steroids binding site. The interaction was hypothesized to serve as an important link between the endocrine, immune and central nervous systems (Su et al., 1988). Recently, new findings identified Sig-1R as a unique chaperone protein that resides in the specific mitochondria associated microdomain (MAM) in the endoplastic reticulum (ER) (Hayashi and Su, 2007). Over the years, Sig-1R has gained its reputation as a multifunctional regulatory protein. For instance, Sig-1Rs play critical roles in CNS diseases and development. Also, Sig-1Rs are well known to exert anti-depressant like effects in vivo (Nakano et al.; Sanchez and Meier, 1997) and in vitro (Nakano et al.; Takebayashi et al., 2002; Tuerxun et al.); pharmacological studies have also shown that Sig-1Rs have therapeutic potential in drug abuse (Cobos et al., 2008; Hayashi et al., 2010; Liu et al., 2005; Romieu et al., 2003; Tsai et al., 2009b) (Hayashi et al.; Nguyen et al., 2005; Stefanski et al., 2004).

Recent studies in neuronal morphological modifications support that Sig-1Rs play pivotal roles in neuroplasticity (Ishima et al., 2008; Takebayashi et al., 2002; Takebayashi et al., 2004; Tsai et al., 2009a) and are related to learning and memory (Meunier et al., 2004;

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Meunier and Maurice, 2004), cognition (Antonini V, 2009; Iyo M, 2008; Kunitachi et al., 2009; Meunier and Maurice, 2004; Takizawa R, 2009), neuroprotection (Goyagi et al., 2003; Katnik et al., 2006; Vagnerova et al., 2006; Yang et al., 2007), drug addiction (Liu et al., 2005; Liu and Matsumoto, 2008), as well as neurodegerative diseases, including Alzheimer's disease, Parkinson's disease, and motor neuron diseases (Luty et al., 2010; Mavlyutov et al., 2010; Meunier et al., 2006; Mishina et al., 2008).

While most of the research focused on how Sig-1Rs relate to CNS disorders based on pharmacological and behavioral studies, the implications of Sig-1R functions in CNS diseases remained vague when considering the underlying cellular mechanisms. Recently, a report indicated that Sig-1Rs could exert their functions from the transcriptional level. Luty and colleagues identified that a nonpolymorphic mutation in the 3′ untranslated region of the *SIGMAR1* gene is the novel causative locus for frontotemporal lobar degeneration and motor neuron disease (Luty et al., 2010). Therefore, characterization of gene expression changes induced by knocking down Sig-1R in primary neurons may help to extend the understanding of Sig-1R's effects on the CNS from the transcriptional level. By combining microarray technology/analysis and Sig-1R gene knockdown using AAV transduction, we have identified several major pathways involving Sig-1R regulation. These findings provide immensely helpful information towards understanding the mechanisms of Sig-1R in the CNS.

Results

Microarray analysis

AAV siSig-1R successfully knock down more than 75% of Sig-1R mRNA expression (Fig 1A) and protein level (Fig 1B) in the neurons. Z test identify siSig-1R-responsive genes that fulfilled a stringent cutoff of $P < 0.05$ and z ratio ≥ 1.5. By these criteria, we further analyzed gene expression patterns using web-based gene ontology (GO) term analysis and the ingenuity pathway database. The GO term analysis revealed that Sig-1R knock down influenced distinct categories of genes related to neuronal development and function, including lipid biosynthesis, synaptic function, cytoskeleton networks, endoplasmic reticulum network and immune responses.

Sig-1R knock down tended to downregulate cytoskeleton network genes, including actin related protein 2/3 complex, subunit 5 (Arpc5), ras-related C3 botulinum toxin substrate 1 (Rac1), and ras homolog gene family member Q (Rhoq). On the other hand, Sig-1R depletion had a propensity for upregulating the actin depolymerization molecule gelsolin (Gsn) as well as other actin filament molecules such as profilin1 (Pfn1), and ezrin (Villin 2).

Our microarray data also indicated that Sig-1Rs significantly impact synaptic functions. Genes that are involved in synaptic transmission, such as Maob, Htr3a and S100b were upregulated in Sig-1R knocked down neurons. Additionally, genes related to synaptic strength were downregulated by siSig-1R, including Gabbr1 (gamma-aminobutyric acid B receptor 1), Gria2 (glutamate receptor, ionotropic, AMPA2) and Sema4f (Semaphorin-4F).

Sig-1Rs are significant regulators for lipid biosynthesis and metabolism, as Sig-1R downregulation strongly inhibited the gene expressions of Scd1 (stearoyl-Coenzyme A desaturase 1), Fdps (farnesyl diphosphate synthetase), Dhcr7 (7-dehydrocholesterol reductase), Fdf1 (farnesyl diphosphate farnesyl transferase 1), Nsdhl (NAD(P) dependent steroid dehydrogenase-like), Sc5d (sterol-C5-desaturase), and Scd2 (stearoyl-Coenzyme A desaturase 2).

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Sig-1Rs have been shown to be involved in augmenting tumor growth and enhancing HIV infection in the presence of cocaine exposure (Roth et al., 2005; Zhu et al., 2003). In addition, Sig-1Rs have been linked to microglial activation and brain injury (Gekker et al., 2006; Hall et al., 2009). Interestingly, Sig-1R knocking down induced potent modulations in genes related to immune responses. In the current microarray data, several immune response genes were significantly upregulated by knocking down Sig-1R, including Oasl2 (2′-5′ oligoadenylate synthetase-like 2), Gbp2 (guanylate nucleotide binding protein 2), Serping1 (serine (or cysteine) peptidase inhibitor, clade G, member 1), Mx2 (myxovirus resistance 2), C1s (complement component 1, s subcomponent), C2 (complement component 2), Psmb8 (proteasome subunit, beta type 8), Irf1 (interferon regulatory factor 1), B2m (beta-2 microglobulin) and Cxcl12 (chemokine (C-X-C motif) ligand 12). Therefore, our data suggested that Sig1R may regulate neuroinflammation via these genes and balance neuron-glia interaction and pro-inflammatory cytokine release.

Quantitative Real-Time RT-PCR analysis

Quantitative real-time RT-PCR analysis *of C1s, Cyba, Prdx6, Nqo1, Rac1, Gstm1, Atf4, Serping1, Maob* and *Ezrin* gene products were performed for siRNA Sig-1R transduced neurons (Fig 2 and table 2). The quantitative real-time RT-PCR results were very similar to those of the microarray data. siRNA Sig-1R transduction decreased the expressions of Rac1 and Atf4 (0.72±0.06 and 0.73±0.06 respectively), increased the expressions of Serping1, C1s, Cyba, Prdx6, Nqo1, Gstm1, Maob and Ezrin (14.31±2.22, 6.05±1.48, 1.54±0.05, 1.62±0.09, 2.3±0.21, 1.37±0.08, 2.1±0.33 and 1.83±0.25, respectively) in the primary neurons.

Discussion

Cytoskeleton networks and synaptic plasticity

The Sig-1R is a critical regulator of neuronal morphogenesis. The expression of genes involved in cytoskeleton organization, including Arp2/3, Gelsolin, Mlc, Rac1, Rho and Profilin, were changed in Sig-1R knockdown neurons. Many studies have highlighted a possible role of Sig-1Rs in neurological diseases and psychological disorders. Our data also showed Sig-1Rs play pivotal roles in regulating synaptic plasticity. As many findings have demonstrated, Sig-1Rs are implicated in neurodegeneration, learning and memory, cognition as well as neuropsychiatric disorders, synapse formation and consequential synaptic strength. Furthermore, cytoskeleton network molecules regulated actin dynamics is involved in shaping dendritic spines and thus contributes to synaptic strength. Imbalance of actin dynamics and the consequent dendritic spine loss are known to impair associative learning (Brigman et al., 2010; Rust et al., 2010). We have previously discovered that Sig-1Rs regulate spine morphology via the Rac GTP pathway (Tsai et al., 2009a); the current data further identified that the genes involved in small GTPase mediated actin cytoskeleton pathway and synapse formation are significantly changed, including Rac, Rho, Arpc5, Mlc, Proflin and Gelsolin. The fact that Sig-1R depletion caused downregulation and inactivation of Rac1 as well as increased actin depolymerization via upregulated Gelsolin explained the importance of Sig-1R in stabilizing spines and synapses. Although Sig-1Rs may not directly regulate Rac and other cytoskeleton molecules (Tsai et al., 2009a), it is of great interest to reveal the importance of Sig-1Rs participating in neuronal morphologies and synaptic plasticity via the regulation of actin dynamics.

Sig-1Rs are key regulators in CNS diseases

Evidence has shown that Sig-1Rs are involved in memory and cognition, and the density of Sig-1Rs in the brain is strongly associated with Alzheimer’s disease (AD). Reports from a postmortem study as well as in vivo brain imaging indicated that low density of Sig-1Rs are

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present in hippocampus (Jansen et al., 1993) and other brain regions in patients with AD (Mishina et al., 2008). The reduced binding potential of Sig-1R in AD patient brains as well as the behavioral studies strengthens the notion that Sig-1Rs have profound impacts on AD pathogenesis. In our data, several genes associated with the pathogenesis of AD are significantly modified in Sig-1R knock down neurons. These include Lrp10 (1.55), Mx2 (10.29), Nqo1 (3.88), Prdx6 (1.60), Rac1 (−1.79), Rhoq (−2.77), Sema4f (−1.53).

Our data also indicated that Sig-1Rs might be associated with CNS degeneration via the regulation of inflammatory responses in the brain. Upon depletion of Sig-1Rs, numerous immune response genes were upregulated in the neuron. Recently, microglia activation induced inflammatory responses in neurodegerative disease and ageing has become increasingly recognized (Gao and Hong, 2008; Hirsch and Hunot, 2009; Kreutzberg, 1996). Evidence from previous studies also supports that inhibition of microglia activation through anti-inflammatory actions protects neurons against degeneration (Liu et al., 2003; Qian et al., 2011; Zhang et al., 2010). A recent report indicated that Sig-1Rs are involved in microglial activation. Hall AA et al found that Sig-1 R agonist 1,3-di-o-tolylguanidine (DTG) significantly suppressed microglia activation. Activation of Sig-1Rs suppressed microglial membrane ruffling, migration, as well as their inflammatory responses. This study indicated that the application of Sig-1R agonist DTG may alleviate neurodegenerative diseases by attenuating the inflammatory responses (Hall et al., 2009). Our current microarray data showed that many microglial activation responsive genes were upregulated upon Sig-1R depletion. For instance, C1s, Irf-1and Serping1 were significantly increased in Sig-1R depleted neurons. Early studies have shown that activation of early complement component C1s is associated with the pathological amyloid cascade(Veerhuis et al., 1996). Additionally, positive staining of complement proteins, including C1s, was observed in AD hippocampal pyramidal neurons (Veerhuis et al., 1999), supporting the possibility that pyramidal neurons expressing complement proteins may be associated with AD pathogenesis (Terai et al., 1997). Furthermore, increased synthesis of complement proteins was found to be associated with microglial activation (Lynch et al., 2004; Schafer et al., 2000). Thus, our data suggested that Sig-1R could potentially be targeted for therapeutic treatments to alleviate chronic neuroinflammation that drives progressive neurdegeneration.

Sig-1Rs balance Oxidative stress in the CNS

Reactive oxygen species (ROS) are acquired in the brain for neuronal differentiation under normal physiological conditions (Tsatmali et al., 2006). However, abnormal production of ROS leads to oxidative stress and cell death. Oxidative stress occurs when there is an imbalance between the production of ROS and the capability of the cells to scavenge for and repair damages from the free radicals and their intermediates. Oxidative stress has been linked to the pathogenesis of a variety of human diseases, and growing evidence has confirmed that an overload of oxidative stress in the CNS is associated with neurological diseases, including aging, Alzheimer’s disease, and Parkinson’s disease.

There are many factors that modulate the oxidative stress responses in the biological system; our recent findings strongly support the critical roles of Sig-1R in regulating free radical productions in the CNS. In a hippocampal neuron model, Sig-1R depletion caused the malfunction of mitochondria, leading to an increase in superoxide anion generation and eventually caused abnormal neuronal morphogenesis (Tsai et al., 2009a). Sig-1Rs are also known to exert their anti-apoptotic actions by regulating bcl-2 expression via the NF-kB pathway (Meunier and Hayashi, 2010). Our current data indicated that Sig-1Rs play a major role mediating the oxidative stress responses. Sig-1R depletion changed the expression of several genes related to the oxidative stress pathway such as Atf-4, Prdx6, Gstp1,2 and Nqo1. Prdx6 is an antioxidant enzyme that belongs to the peroxiredoxin family. A recent finding showed that the Prdx6 expression level is very low in neurons but higher in glia

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populations in healthy brain tissue; however, in brain tissues of patients with AD, there was an increase number in Prdx6 positive astrocytes. It was suggested that oxidative stress is also involved in the progression of AD and Prdx6 can provide defense in this regard (Power et al., 2008). Our data showed when neurons expressed lower levels of Sig-1R, antioxidant enzymes, such as Prdx6, were upregulated, suggesting that depleting Sig-1R jeopardized neuronal conditions and possibly initiated antioxidant defense responses that require astrocytic activation.

Sig-1R knock down also induced a family of glutathione S-transferase (GST) enzyme upregulation, including Gstm1 (2.89), Gstp1 (2.31) and Gstp2 (1.54). The GST superfamily contains important enzymes that play a role in the detoxification of lipid peroxidation products in response to oxidative stress. GST isoenzyme expression is also associated with oxidant generation related aging in rat cerebral cortex and cerebellum (Martinez-Lara et al., 2003). The current microarray data showed that when neurons went through Sig-1R deficits many antioxidant genes were upregulated. These data suggested Sig-1R deficiency caused cell damage, thus indicating that the receptors might elicit mechanisms to compensate for oxidative stress and could perhaps act as a front-line defense system to delay cell death.

Sig-1R is a major character in Lipid metabolisms

Lipids are essential for structuring cellular compartments and play a major role in brain development. The genes in lipid metabolism were greatly affected by Sig-1R knocking down. In general, lipid biosynthesis was seriously hampered in the absence of Sig-1Rs. The microarray data aided in identifying candidate genes involved in brain development.

We observed a reduction in activating transcription factor 4 (Atf-4) gene expression in Sig-1R knocked down neurons. Atf-4 is well known as a transcriptional factor involved in propagating the death response to oxidative stress in the CNS. An overload of oxidative stress usually leads to induction of Atf-4. Here in our microarray data, the Atf-4 expression was decreased rather than increased. Interestingly, in addition to its role in response to oxidative stress, recent data also supports the relevance of Atf-4 in regulating lipid metabolism and thermogenesis (Wang et al., 2010). It has been documented that Atf-4 deficient mice are lean and associated with increased energy expenditure and lipolysis. These findings further imply that Sig-1Rs may participate in lipid metabolism via regulating Atf-4 transcriptional activities.

Additionally, since Sig-1Rs mainly reside in the MAM and regulate many signaling cascades within, and lipid importation into mitochondria from ER most likely involves the MAM, it is possible that Sig-1Rs play a critical role in tethering the connection between mitochondria and the ER to stabilize the MAM microdomain. An emerging concept noted MAM dysfunction could be a major contribution to certain lipid metabolism related neurological diseases such as Alzheimer’s disease, thus the critical functions of Sig-1R in lipid biosynthesis and metabolism should not be overlooked.

Conclusions

The microarray data indicated that Sig-1Rs are involved in a wide spectrum of molecular pathways in neurons. The results of the microarray study provide information that can direct our attention to previously unknown genes for further analysis to decipher their potential functions in Sig-1R related physiological and pathophysiological conditions. Herein, we identified several major genes that are responsible for oxidative stress caused by Sig-1R depletion in the neurons. The microarray analysis also provides the functions and pathways linked to Sig-1R regulated neuronal functions. We found Sig-1R depletion not only leads to

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a reduction in lipid biosynthesis but also to an increase in lipid catabolic processes. Sig-1R depletion also initiates strong immune responses in the neuron.

In agreement with our previous findings, Sig-1Rs are involved in actin organization in the dendritic spines and can affect synaptic functions within (Tsai et al., 2009a). These results suggest that with a deficiency of Sig-1Rs, oxidative stress strikes neurons and affects lipid metabolism, immune response, the actin cytoskeleton network, the ER membrane, as well as synaptic functions, thus placing neurons in a harsh condition that is prone to the pathogenesis of CNS diseases.

Methods

Illumina oligonucleotide microarray and data analysis

Using the Illuminal TotalPrep RNA Amplification Kit (Ambion; Austin, TX, cat # IL1791), 0.5 μg of total RNA was used to generate biotin labeled cRNA. Using an oligo-dT primer containing the T7 RNA polymerase promoter site, total RNA was converted into single-stranded cDNA with reverse transcriptase, and then copied to produce double-stranded cDNA. The double-stranded cDNA was next cleaned and concentrated with the columns included in the amplification kit, then used in an in vitro transcription reaction overnight to generate single-stranded cRNA that was labeled via the incorporation of biotin-16 UTP.

An Illumina Bead Arrays RatRef-12 Expression BeadChip (Illumina, Inc.; San Diego, CA, cat # BD-27-303) was used to carry out the microarray labeling. A total of 0.75ug of biotin-labeled cRNA was hybridized at 58 °C for 16 hours to Illumina Bead Arrays RatRef-12 Expression BeadChip. There are 24,000 well-annotated RefSeq transcripts with approximately 30-fold redundancy in each BeadChip. The arrays were washed and then blocked, then, after staining with streptavidin-Cy3, the labeled cRNA was detected. The arrays were scanned using an Illumina BeadStation 500X Genetic Analysis Systems scanner and the image data extracted using Illumina BeadStudio v3.0 or GenomeStudio v1.0 software.

The specifics of the microarray protocol and the data analysis were carried out as described by previous literature (Stranahan et al., 2010). Gene ontology (GO) was used to analyze the gene lists. PAGE (Parametric Analysis of Gene Set Enrichment) was used for the GO analysis with all of the genes on the microarray (Kim and Volsky, 2005). For each GO term, the z score represents an encompassing summary of all the members under each term. The changing p values for each GO term were calculated as an indication of significant changes, with a cutoff of a probability (p) value less than or equal to 0.05.

Data analysis

DIANE 6.0, a spreadsheet-based microarray analysis program based on the SAS JMP7.0 system, was used to analyze the microarray data. The raw microarray data were filtered using Z normalization and by the detection p-value, then tested further for significant changes as described previously. The quality of the samples was first analyzed using scatter plots, gene sample z scores based hierarchy clustering to exclude possible outliers, and principal component analysis. ANOVA tests were used to identify and eliminate genes with greater variances within each comparing group. After calculating the Z ratio, it was determined that genes were differentially expressed, indicating the fold-difference between experimental groups and the false discovery rate (fdr), which is a compensation for the expected proportion of falsely rejected hypotheses. Individual genes with p value ≤ 0.05, absolute value of Z ratio ≥ 1.5 and fdr ≤ 0.3 were considered significantly changed.

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To identify clustering within groups, hierarchy/K-means clustering and Principal Components Analysis (PCA) were performed. The array data for each animal used in the experiment was also originally hierarchically clustered in Ilumina Bead Studio version 2.0. All results were presented graphically as well. For gene set enrichment analysis, the PAGE algorithm was applied by using all genes in each sample as input to compare against the data set supplied by the MIT Broad Institute and the Gene Ontology Institute. The lists of differentially expressed genes and Z ratios were entered into the PAGE Pathway Analysis software to organize them according to known biological pathways for each relevant comparison. For each functional grouping, the enrichment z scores were calculated based on the chance of mRNA abundance changes predicting these interactions and networks by z-test.

To calculate the P-value, the number of user-specified genes of interest participating in a given function or pathway was compared to the total number of occurrences of these genes in all functional/pathway annotations stored in the knowledge base. The Pathways must have had at least three genes found in the microarray gene set. The cutoff criteria for the significant pathway/Gene Ontology selection was a p value ≤ 0.05 and fdr ≤ 0.3. The pathway/Gene Ontology results are further presented by cluster heat map for their association relations and by bar plot for their change patterns. Also supplied are the related gene sets in each pathway as well as their expression patterns and the related pathway.

The genes in the pathway/functional groups are also supplied with their expression patterns indicated. In addition, the pathway and gene ontology information for each gene are available. To identify top network functions involving differentially expressed genes, Ingenuity Pathways Analysis IPA (Ingenuity Systems; Redwood City, CA) and/or Pathway Studio 6.1 were used. The network analysis utilizes a database of known functional interactions and protein functions to algorithmically infer biochemical interactions. The right-tailed Fisher's Exact Test was used to calculate the significance of functions and pathways. The type of protein products and protein-protein interactions is available with the request and is presented graphically by HTML linkage hot maps produced by Pathway Studio 6.1. Their literature references and cellular location are also supplied for each protein-protein interaction linkage.

Quantitative Real-Time RT-PCR

Quantitative Real-Time RT-PCR was performed and analyzed using Roche LightCycler ® instrument 480 II. PCR reactions were carried out in a total volume of 20 μL in PCR mix containing 10 μL 2X LightCycler ® probe master, 500nM reference gene primer (GAPDH), 500 nM each of sense and antisense primer, 100 nM each of target gene probe and reference gene probe, and 5 μL of 100ng cDNA filled up to 20 μL with DEPC-treated H_2O. To normalize the cDNA content of the samples, we used the comparative threshold ($\Delta\Delta C_T$) cycle method, which consists of the normalization of the number of target gene copies versus the endogenous reference gene, GAPDH. The CT is defined as the fractional cycle number at which the fluorescence generated by cleavage of the probe passes a fixed threshold baseline when amplification of the PCR product is first detected.

Primers and probes for RT-PCR

The primers and probes were designed using Universal Probe Library Assay Design Center (Roche). Primer sequences and probes are listed in Table 3.

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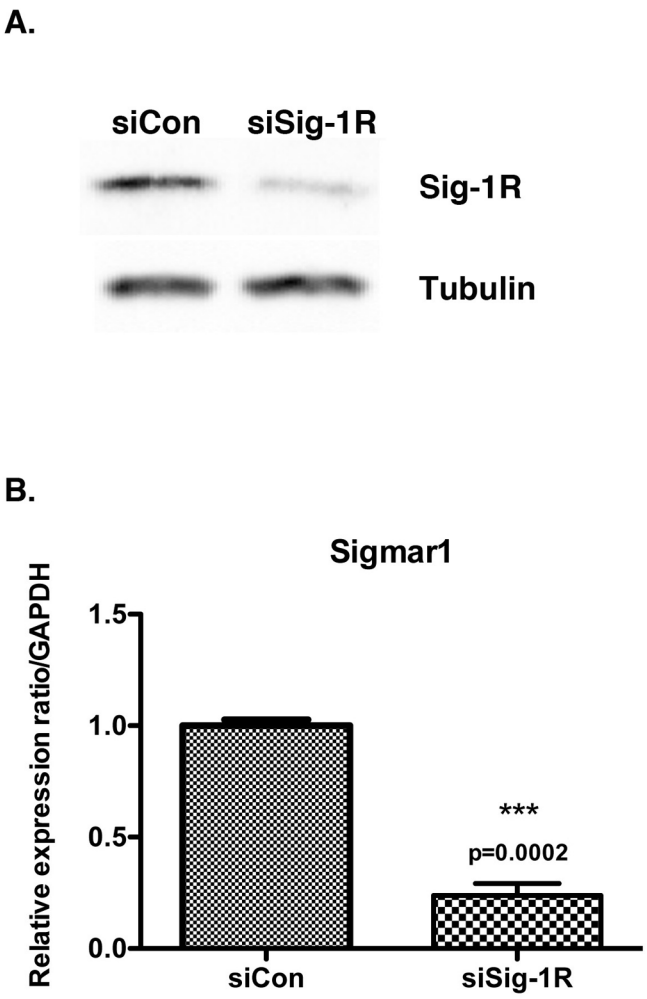

Figure 1.

AAV transduction of siRNA specific for Sig-1R reduces Sig-1R mRNA and protein levels. Primary cultured E14 neurons were transduced with *sigmar1* siRNA or control siRNA. Total protein and mRNA was purified after transduction and analyzed by (A) western blotting and (B) qRT-PCR, respectively. N=6.

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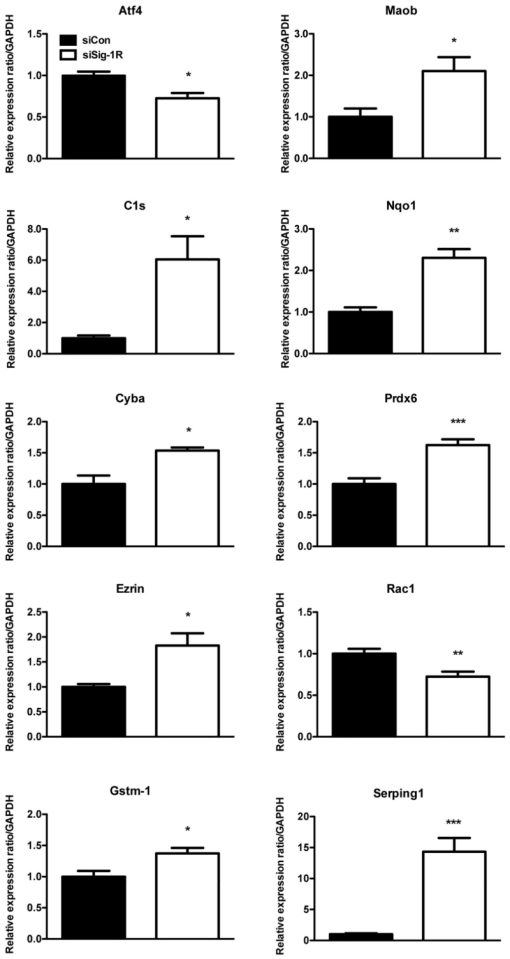

Figure 2.

Real time quantitative PCR data on selected genes. Each bar represents mean±SEM, n=6. *p<0.05, **p<0.01, ***p<0.001 compared to each control.

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TABLE I

Gene expression changes in Sig-1R knockdown neurons.

Gene Symbol	Description	Z Ratio	Accession Number
	Actin cytoskeleton organization and biosynthesis		
Gsn	gelsolin	4.63	NM_001004080.1
Pfn1	profilin 1 (Pfn1), mRNA.	2.66	NM_022511.2
Mlc1	megalencephalic leukoencephalopathy with subcortical cysts 1 homolog	2.40	XM_235558.4
Cryab	crystallin, alpha B (Cryab), mRNA.	2.19	NM_012935.2
Rhob	ras homolog gene family, member B	2.05	NM_022542.1
Ezr	ezrin (villin 2)	1.89	NM_019357.1
Mk1	Mk1 protein	1.77	NM_134399.2
Arpc5	actin related protein 2/3 complex, subunit 5	−1.79	NM_001025717.1
Rac1	ras-related C3 botulinum toxin substrate 1	−1.79	NM_134366.1
Rhoq	ras homolog gene family, member Q	−2.77	NM_053522.1
	Synaptic transmission		
Maob	monoamine oxidase B (Maob), nuclear gene encoding mitochondrial protein	5.83	NM_013198.1
Pnoc	prepronociceptin	2.71	NM_013007.1
Htr3a *	5-hydroxytryptamine (serotonin) receptor 3a	2.29	NM_024394.1
S100b	S100 protein, beta polypeptide, neural	2.12	NM_013191.1
Bat2	HLA-B associated transcript 2	2.04	NM_212462.1
Agrn	agrin	1.97	NM_175754.1
Tmod2	tropomodulin 2	−1.65	NM_031613.1
Gp1bb	glycoprotein Ib, beta polypeptide	−1.69	NM_053930.3
Gria2 *	glutamate receptor, ionotropic, AMPA2	−1.73	NM_017261.1
	Synapse and postsynaptic membrane		
Htr3a *	5-hydroxytryptamine (serotonin) receptor 3a	2.29	NM_024394.1
Slc32a1	solute carrier family 32 (GABA vesicular transporter), member 1	1.59	XM_001068818.1
Sema4f	sema domain, immunoglobulin domain, transmembrane domain and short cytoplasmic domain, 4F	−1.53	NM_019272.1
Lzts1	leucine zipper, putative tumor suppressor 1	−1.69	NM_153470.1
Gria2 *	glutamate receptor, ionotropic, AMPA2	−1.73	NM_017261.1
Gabbr1	gamma-aminobutyric acid (GABA) B receptor 1	−1.74	NM_031028.2
	Lipid biosynthesis and catabolic process		
Pla2g7	phospholipase A2, group VII (platelet-activating factor acetylhydrolase, plasma)	4.85	NM_001009353.1
Lamb2	laminin, beta 2 (Lamb2), mRNA.	2.51	NM_012974.1
Plcd1	phospholipase C, delta 1	1.91	NM_017035.1
Prdx6 *	peroxiredoxin 6	1.60	NM_053576.1
Scd2	stearoyl-Coenzyme A desaturase 2	−1.59	NM_031841.1
Acly	ATP citrate lyase (Acly)	−1.82	NM_016987.1
Sc5d	sterol-C5-desaturase	−1.95	NM_053642.2
Idi1	isopentenyl-diphosphate delta isomerase	−2.39	NM_053539.1
Nsdhl	NAD(P) dependent steroid dehydrogenase-like (Nsdhl), mRNA.	−2.55	NM_001009399.1

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Gene Symbol	Description	Z Ratio	Accession Number
Fdft1*	farnesyl diphosphate farnesyl transferase 1 (Fdft1), mRNA.	−2.72	NM_019238.2
Dhcr7*	7-dehydrocholesterol reductase (Dhcr7), mRNA.	−2.89	NM_022389.2
Sc4mol*	sterol-C4-methyl oxidase-like	−2.96	NM_080886.1
Hmgcr*	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	−3.01	NM_013134.2
Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	−3.14	NM_017268.1
Fdps	farnesyl diphosphate synthetase	−3.17	NM_031840.1
Mvd	mevalonate (diphospho) decarboxylase	−3.67	NM_031062.1
Scd1*	stearoyl-Coenzyme A desaturase 1	−6.19	NM_139192.1
	Endoplasmic reticulum		
Irgm	immunity-related GTPase family, M	6.26	NM_001012007.1
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1	2.65	XM_342218.3
Ptgfrn	prostaglandin F2 receptor negative regulator	1.86	NM_019243.1
Resp18	regulated endocrine-specific protein 18	1.74	NM_019278.1
Cyp1b1	cytochrome P450, family 1, subfamily b, polypeptide 1	1.61	NM_012940.1
Napg	N-ethylmaleimide-sensitive factor attachment protein, gamma	−1.50	XM_225881.4
Scd2	stearoyl-Coenzyme A desaturase 2	−1.59	NM_031841.1
Calu	calumenin (Calu), transcript variant 2, mRNA.	−1.66	NM_001033898.1
Sc5d	sterol-C5-desaturase	−1.95	NM_053642.2
Srebf1	sterol regulatory element binding factor 1	−2.62	XM_213329.4
Fdft1*	farnesyl diphosphate farnesyl transferase 1 (Fdft1), mRNA.	−2.72	NM_019238.2
Dhcr7*	7-dehydrocholesterol reductase (Dhcr7), mRNA.	−2.89	NM_022389.2
Sqle	squalene epoxidase	−2.93	NM_017136.1
Sc4mol*	sterol-C4-methyl oxidase-like	−2.96	NM_080886.1
Ptp4a1	protein tyrosine phosphatase 4a1	−2.99	NM_031579.1
Hmgcr*	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	−3.01	NM_013134.2
Scd1*	stearoyl-Coenzyme A desaturase 1	−6.19	NM_139192.1
	Oxidative stress response		
Nqo1	NAD(P)H dehydrogenase, quinone 1	3.88	NM_017000.2
Gstm1	glutathione S-transferase, mu 1	2.89	NM_017014.1
Stat3	signal transducer and activator of transcription 3	2.76	NM_012747.2
Vcam1	vascular cell adhesion molecule 1 (Vcam1), mRNA.	2.38	NM_012889.1
Cyba	Rattus norvegicus cytochrome b-245, alpha polypeptide (Cyba), mRNA.	2.01	NM_024160.1
Aox1	aldehyde oxidase 1	2.20	NM_019363.2
Prdx6*	peroxiredoxin 6	1.60	NM_053576.1
Usp14	ubiquitin specific peptidase 14	−1.63	NM_001008301.1
Atf4	activating transcription factor 4	−2.23	NM_024403.1
	Immune response		
Oasl2	2′-5′ oligoadenylate synthetase-like 2	13.85	NM_001009682.1
Gbp2	guanylate nucleotide binding protein 2	13.27	NM_133624.1
Serping1	serine (or cysteine) peptidase inhibitor, clade G, member 1	10.58	NM_199093.1

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Gene Symbol	Description	Z Ratio	Accession Number
Mx2	myxovirus (influenza virus) resistance 2	10.29	NM_134350.2
Mx1	myxovirus (influenza virus) resistance 1	7.92	NM_173096.2
C1s	complement component 1, s subcomponent	7.50	NM_138900.1
C2	complement component 2	6.91	NM_172222.2
Psmb8	proteasome (prosome, macropain) subunit, beta type 8 (large multifunctional peptidase 7)	6.58	NM_080767.1
C1r	complement component 1, r subcomponent	5.85	XM_242644.4
Sp110	SP110 nuclear body protein	5.53	NM_001034137.1
Psmb9	proteosome (prosome, macropain) subunit, beta type 9	5.29	NM_012708.1
Irf1	interferon regulatory factor 1	4.09	NM_012591.1
B2m	beta-2 microglobulin	2.96	NM_012512.1
Psme1	proteasome (prosome, macropain) 28 subunit, alpha	2.60	NM_017264.2
Cxcl12	chemokine (C-X-C motif) ligand 12 (Cxcl12), transcript variant 1	2.24	NM_022177.3
Orai1	ORAI calcium release-activated calcium modulator 1	1.65	NM_001013982.1

*Indicates same gene appears in other pathways

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TABLE II

Comparison between quantified values from the microarray and quantitative real-time RT-PCR in Sig-1R knockdown neurons.

Gene Name	Methods	
Atf4	Z ratio by Microarray	−2.23
	Relative expression by RT-PCR	0.73±0.06 *
C1s	Z ratio by Microarray	7.5
	Relative expression by RT-PCR	6.05±1.48 *
Cyba	Z ratio by Microarray	2.01
	Relative expression by RT-PCR	1.54±0.05 *
Ezrin	Z ratio by Microarray	1.89
	Relative expression by RT-PCR	1.83±0.25 *
Gstm1	Z ratio by Microarray	2.89
	Relative expression by RT-PCR	1.37±0.08 *
Maob	Z ratio by Microarray	5.51
	Relative expression by RT-PCR	2.1±0.33 *
Nqo1	Z ratio by Microarray	3.88
	Relative expression by RT-PCR	2.3±0.21 **
Prdx6	Z ratio by Microarray	1.6
	Relative expression by RT-PCR	1.62±0.09 ***
Rac1	Z ratio by Microarray	−1.79
	Relative expression by RT-PCR	0.72±0.06 **
Serping1	Z ratio by Microarray	10.58
	Relative expression by RT-PCR	14.31±2.22 ***

*$p<0.05$,

**$p<0.01$,

***$p<0.001$ compared to each control.

See “Methods” for detailed calculations of “Z ratio by Microarray”.

“Relative expression by RT-PCR” represents ratios of Sig-1R knockdown neurons to respective controls.

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TABLE III

Probes and qRT-PCR primers of target genes.

Primers	Sequences. Sense, anti-sense (5′-3′)	Probes
Atf4	ggcgtattagaggcagcaga, ctgctgggtttcgtgaagag	#116
C1S	tcacggcatgaccagaag, ccagggctcagtgtcacc	#75
Cyba	gccattgccagtgtgatcta, ctcaatgggagtccactgct	#46
Ezrin	cagccgacttaccgaggac, gtcacgcggacgttgatt	#21
Gapdh	ctgcaccaccaactgcttag, tgatggcatggactgtgg	Rat Gapdh
Gstm1	tgttacaaccccgactttga, tcttctcagggatggtcttca	#106
Maob	aatgcgatgtgatcgtggt, ggccacagtcatgcaaaag	#66
Nqo1	agcgcttgacactacgatcc, caatcagggctcttctcacc	#50
Prdx6	ccacgggcagaaactttg, ccatcacactctctcccttctt	#78
Rac1	catcagttacacgaccaatgc, cattggcagaatagttgtcaaaga	#80
Serping1	ccaaaggtgtcacttctgtgtc, cataggtgtcccgtatggcta	#26
Sigmar1	aaagtgaggtctattacccaggag, tttggtccccactccaca	#80