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Original Article

Versican isoforms modulate expression and function of nicotinic acetylcholine receptors

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Abstract: Versican is a chondroitin sulfate proteoglycan whose isoforms are differentially expressed, but little is known of their functions in the neuronal system. Here we show that isoforms of versican play different roles in neuronal differentiation and neurite outgrowth. Expression of versican V1 isoform in PC12 cells induced complete neuronal differentiation and increased the expression of nicotinic acetylcholine receptor in NGF-independent manners. The V1-induced neuronal differentiation was different from the NGF-induced differentiation, showing a specific profile of nAChR subunit expression and distinct kinetics of receptor-gated channel activity. Our results have implications for understanding how versican regulates neuronal development, function and repair.

Key words: Differentiation, neurite outgrowth, phosphorylation, PC12 cells

Introduction

Versican is an extracellular chondroitin sulfate proteoglycan detected in developing and mature brains. This proteoglycan was originally isolated from developing limb buds in the chicken [1], and was detected in the normal human central nervous system and in brain tumors [2]. Structurally, versican is made up of an N-terminal G1 domain, a chondroitin sulfate (CS) attachment region, and a C-terminus containing a selectin-like (or G3) domain [3]. The latter contains two epidermal growth factor (EGF)-like repeats, a lectin-like motif (also known as carbohydrate recognition domain or CRD) and a complement binding protein (CBP)-like motif [1,4,5]. The CS attachment region is encoded by two exons producing CSα and CSβ domains. Due to alternative splicing, four isoforms (V0, V1, V2 and V3) may be generated [6,7]. Versican is known to associate with a number of other molecules in the ECM including hyaluronan, tenascin, fibulin-1, fibrillin, fibronectin, CD44 and selectins [3].

Versican is one of the major extracellular molecules in developing and mature brains, but how this molecule affects neuronal differentiation, development and maturation is unknown. Studies of brain development and maturation have shown that versican V1/V0 and V2 have complementary expression patterns [8,9]. While versican V1/V0 is mainly expressed in the late stage of embryonic development [10], versican V2 becomes a major chondroitin sulfate proteoglycan in the mature brain [11]. Versican V3, which differs from the other isoforms in that it contains no modification site for GAG chains, plays a role

The abbreviations used are: ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, EGF receptor; NGF, nerve growth factor, DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; PAGE, polyacrylamide gel electrophoresis; G3, selectin-like domain; GAG, glycosaminoglycan; AChR, acetylcholine receptor.

in cell adhesion and spreading. It is also associated with reduced migration and cell proliferation in endothelial cells [12]. These results suggest that differences in the CS domains may underlie differences in function among versican isoforms.

Our previous studies have demonstrated that different versican domains play different roles in regulating cellular activities [13-18]. To investigate the effect of versican on neuronal differentiation and neurite outgrowth, we have cloned and expressed the full-length versican V1 and V2 isoforms in PC12 cells, an extensively-used cell line for studying neuronal differentiation [19,20]. These cells respond to nerve growth factor (NGF) and other stimuli, shifting from a chromaffin cell-like phenotype to a neurite-bearing sympathetic neuron-like phenotype [21]. Specifically, differentiated neuron-like PC12 cells characteristically express neuronal nicotinic acetylcholine receptors (AChR) [22]. In the present report, we studied the effect of versican levels on nAChR expression, in part from the perspective of nAChR as ideal targets for regulatory influences. Here, we show that versican V1 induces neuronal differentiation and promotes neurite outgrowth. The V1-induced neuronal differentiation is independent of NGF signaling and displays a unique profile of AChR subunit expression.

Materials and methods

Materials and cell cultures

Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, neurobasal medium, B27 supplement, N2 supplement, MEM, sodium pyruvate, FBS, trypsin/EDTA and Geneticin (G418) were purchased from Invitrogen. Antibodies against nAChR isoforms 3, 4, 7, and 2 were purchased from Santa Cruz Biotechnology. NGF, goat anti-mouse-FITC, horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody, poly-D-lysine, poly-L-lysine and all chemicals were purchased from Sigma (St. Louis, MO). ECL Western blot detection kit was from Amersham. Tissue culture plates were from Nunc Inc. TRITC-labeled phalloidin was from Molecular Probes. The PC12 cell line was from the American Type Culture Collection (Rockville, MD).

Construct generation and expression

To study the effect of versican on neuronal differentiation, two constructs were used, including versican V1 [22] and V2 isoforms [22]. To generate versican V2 construct, the CS fragment in the mini-versican was replaced with CS domain, which was generated by PCR using two primers pCS1NMluI and pCS1CXhoI and chicken genomic DNA as the template. The PCR product was digested with restriction endonucleases MluI and XhoI. The PCR product, combined with EcoRI-MluI-digested G1 domain (from the mini-versican), was inserted into EcoRI-XhoI-digested G3 domain-containing vector (pcDNA3, obtained from the mini-versican construct) to generate the V2 construct. These constructs were stably expressed in PC12 cells using Lipofectamine according to the manufacturer's instructions. Gene expression was confirmed by Western blotting as described [23].

To study the function of a siRNA expression construct, it is essential to be able to monitor the siRNA-transfected cells in a real-time manner. To accelerate research progress, we have engineered an siRNA expression vector containing green fluorescent protein (GFP) element. This plasmid, named pN1-Mod, contains a Bluescript backbone, a neomycin-resistant unit, a siRNA expression element, and a CMV promoter driving GFP. The structure of the plasmid has been described recently [24,25]. To generate siRNA constructs targeting the V1 construct expressed in PC12 cells, several target sequences (including nucleotides 5337-5355, gcctgacatgactgcttct, nucleotides 9151-9170, gaggttagttctgatatgg, and nucleotides 10789-10808, cactaccatc-gctggatca) of chicken versican was inserted into the pN1-Mod plasmid. After DNA sequencing, the silencing effects of these siRNA constructs were analyzed. One of them, targeting the nucleotides 5337-5355 located in the G3 domain of versican, was used for this study. The silencing effect of the siRNA construct on G3 expression was analyzed by Western blot. We have previously used this technique to effectively silence the versican expression [26-28].

Cell differentiation

Confluent PC12 cells were starved in RPMI 1640 medium containing 1% serum for 24 h. The cells were harvested and 1 x 10^5 cells were seeded on 6-well plates in RPMI 1640 medium containing 1% serum, and incubated

at 37 ^{o}C for 14 d. Neurite outgrowth was measured and photographed. As well, 1 x 10^5 cells were seeded on plastic (uncoated), or collagen type I (10 μg/ml)- or laminin (20 g/ml)-coated 6-well plates in serum-free RPMI 1640 medium. Starved PC12 cells (1 x 10^5 cells) were also seeded on collagen type I-coated 6-well plates in RPMI 1640 medium containing 1% serum in the presence or absence of 50 ng/ml NGF and incubated at 37 ^{o}C for 7d, to which 50 ng/ml NGF was added every other day as previously reported [29]. Studies were conducted in triplicate using 3 different clones. Results obtained from one typical clone were provided.

Immunofluorescence

PC12 cells were grown on type I collagen-coated glass coverslips in RPMI 1640 medium containing 1% serum for 16-48 h, fixed with 4% paraformaldehyde, and blocked with 1% bovine serum albumin (BSA). To detect extracellular and cell surface binding proteins, the cells were first stained with 4B6 monoclonal antibody overnight at 4°C, washed with PBS, and then permeabilized in PBS containing 0.2% Triton X-100. The cells were then incubated with TRITC-labeled phalloidin for 1.5 h at room temperature, washed, and incubated with FITC-conjugated anti-mouse Ig antibody. To examine intracellular protein expression, the cultures were first permeabilized in PBS containing 0.2% Triton X-100 and then stained with 4B6 monoclonal antibody overnight at 4°C. After washing with PBS, the cells were incubated with TRITC-labeled phalloidin and FITC-conjugated anti-mouse Ig antibody for 1.5 h at room temperature. After the final wash and mounting, the cells were examined using a laser confocal microscope with a 60× objective. Using this technique, we have successfully monitored neuronal cellular functions [29,30].

Western blot for AChRs

Acetylcholine receptors (AChRs) in PC12 cells stably expressing versican V1, V2 and control vector were analyzed by Western blot probed with anti-AChR beta 2, alpha3, 4 and 7 antibodies (Santa Cruz Biotechnology). Equal numbers of cells from confluent culture were seeded in 1% serum (0.5% FBS and 0.5% horse serum) containing RPMI 1640 medium and incubated for 24 h. Cells in both suspension and adhesion were lysed and equal amounts of the lysate were loaded for analysis on 7-10% SDS gels. After transfer, membranes were probed with anti-AChR beta, alpha 3, 4 and 7 antibodies, respectively, at 4°C overnight, followed by incubation with biotinated anti-goat Ig at room temperature for 1.5 h, washes, and streptavidin-HRP incubation at room temperature for 1.5 h.

Electrophysiology

The versican- and vector-transfected cells were used for whole-cell recordings at room temperature with an Axopatch-1D amplifier (Axon Instruments). Recording electrodes (3-4 MΩ) were constructed from thin walled glass (1.5 mm diameter; World Precision Instruments) using a two-stage puller (PP-830, Narishige). The extracellular solution was composed of (in mM) 145 NaCl, 1.3 $CaCl_2$, 5.4 KCl, 25 HEPES, 33 glucose, pH 7.4 and osmolarity between 315 and 335 mOsm. The intracellular solution in the recording electrode consisted of (in mM) 155 KCl, 10 Hepes, 2 $MgCl_2$, 1 $CaCl_2$, 2 tetraethylammonium, 4 K-ATP, pH 7.35, and osmolarity at 310 mOsm. The cells were voltage-clamped at -60 mV. Rapid application of the acetylcholine receptor agonist carbachol (CCh, 250 μM) to the cell was achieved by means of a computer-controlled multibarrel perfusion system (Warner). The evoked electrical signal was digitized, filtered (1 kHz), and acquired on-line using the program pClamp (Axon Instruments). The amplitude of CCh-induced currents was measured by the software of Clampfit (Axon Instruments). Data were analyzed and plotted using the SigmaStat software (Jandal Scientific Co). A p value < 0.05 was considered as significant.

Results

The role of versican V1 in neuronal differentiation and neurite outgrowth is NGF-independent

We have previously demonstrated that versican V1 and V2 isoforms play important roles in neuronal differentiation [29]. Here we continued on these studies to obtain further details of the roles of versican V1 and V2 isoforms in mediating neurite growth. Two versican full-length isoforms (V1 and V2) were used as described previously (**Figure 1A**). Both constructs contain either the full-length

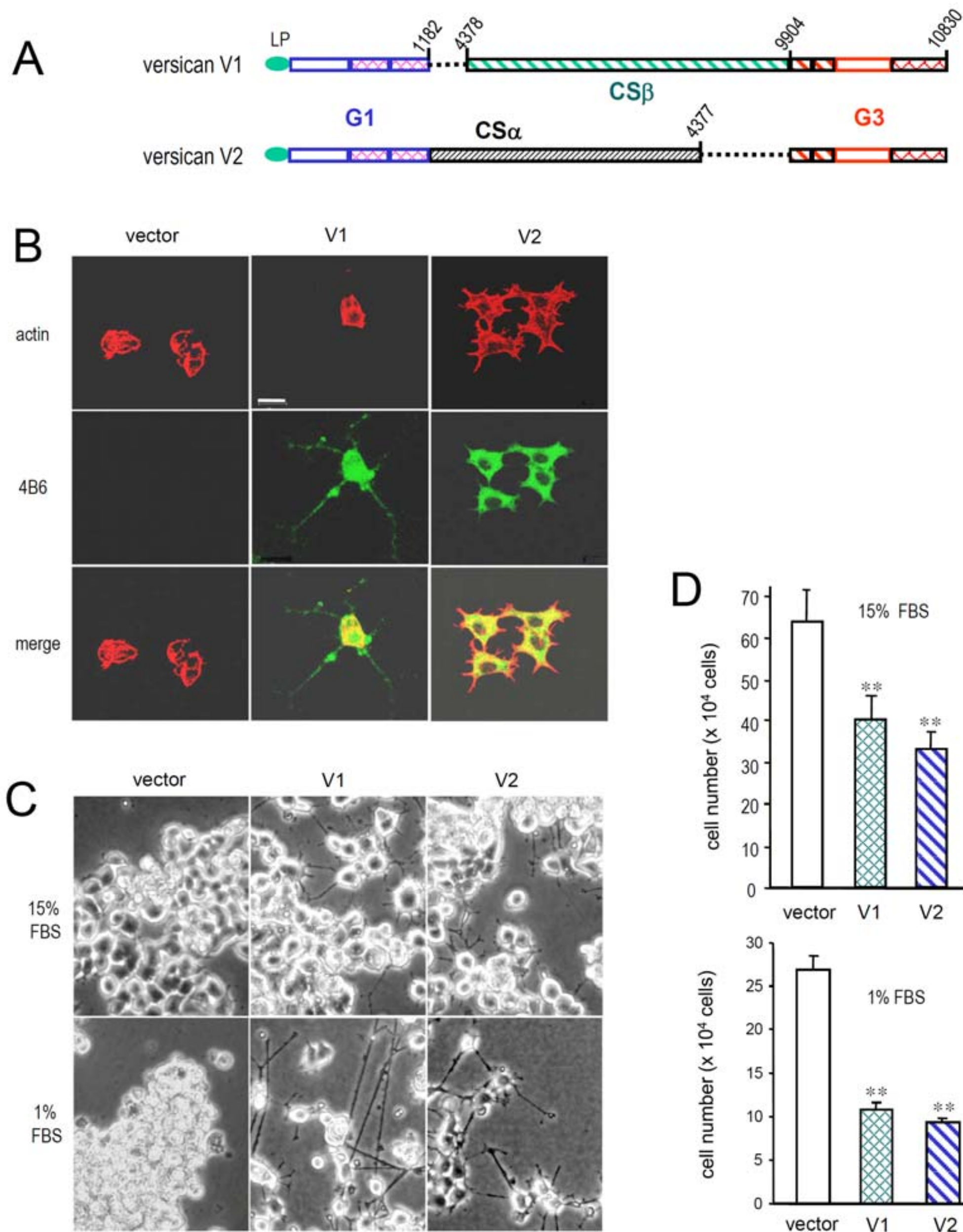

Figure 1. Expression of versican V1 isoform induces PC12 cell differentiation and neurite outgrowth. A, Diagram of the structures of versican V1 and V2 constructs. B, Stable expression of V1 and V2 in PC12 cells was analyzed by immunocytometry probed with phalloidin (for actin staining) and a monoclonal antibody that recognizes an epitope engineered in both V1 and V2 constructs. Scale bar, 10 µm. C, Vector-, V1- and V2-transfected PC12 cells were maintained on tissue culture plates in RPMI 1640 medium containing 1% or 15% serum. In the presence of 15% serum, versican V1-, V2-transfected cells exhibited low levels of differentiation, showing extension of processes, but no differentiation was detected in vector-transfected cells. When these cells were maintained in 1% serum/RPMI 1640 medium, only versican V1-transfected cells exhibited sustained differentiation and neurite outgrowth. D, Vector-, V1- and V2-transfected PC12 cells were maintained on tissue culture plates in culture medium RPMI 1640 containing 15% or 1% serum. The effect of these two constructs on PC12 cell proliferation was determined by cell count. V1-, and V2-transfected cells exhibited a significantly reduced rate of proliferation compared to the vector-transfected cells (n=3, ** p< 0.01). Each data point represented the mean ± s.e.m.

versican V1 or V2 isoforms and the leading peptide of chicken link protein, which harbors an epitope recognized by the monoclonal antibody 4B6 [5,29,31]. Expression of the V1

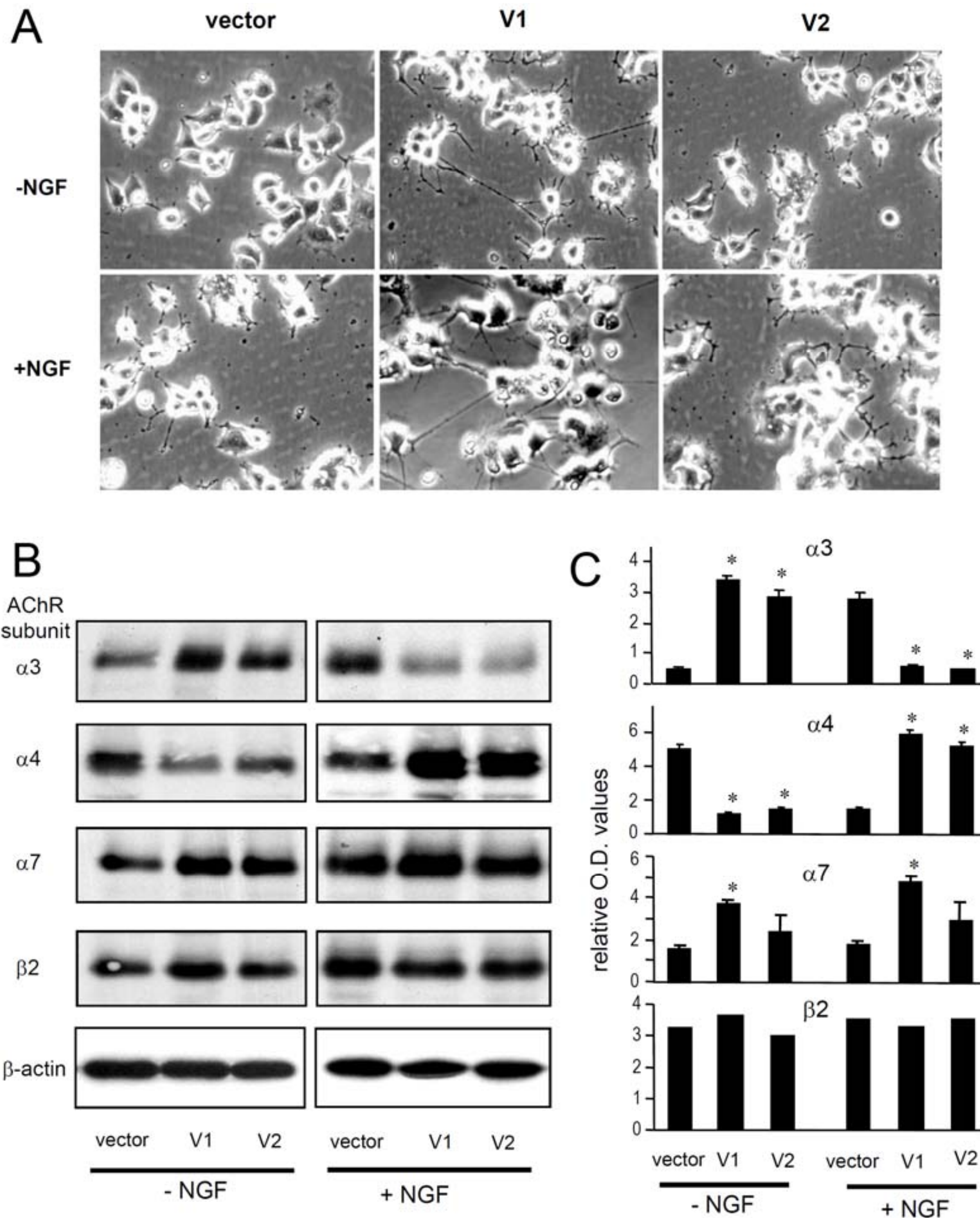

Figure 2. Versican alters the expression of nicotinic receptors. **A,** PC12 cells stably transfected with vector or V1 and V2 constructs were incubated with NGF (50 ng/ml). Four days after treatment photographs of differentiation were acquired. **B,** Western blot analyses of AChR subunits α3, α4, α7 and α2 in the presence or absence of NGF. Expression of α3 and α7 was up-regulated by V1- and V2-transfection, while expression of α4 was down-regulated. In the presence of NGF, expression of α3 and α4 exhibited complementary patterns. **C,** The intensities of the protein bands were scanned, and the densitometry results were semi-quantified. (n = 3, * $p < 0.05$).

and V2 constructs was confirmed by immunocytometry, probed with 4B6 monoclonal antibody and anti-actin antibody (**Figure 1B**). The experiments indicated that both constructs were well expressed in the selected cell lines.

We have previous demonstrated that PC12 stably expressing V1 and V2 isoforms of versican altered in cell proliferation and morphology [29]. The effect of these two isoforms was examined in more detail. When the cells were maintained in medium

containing 15% serum, V1-, V2-transfected cells exhibited slower growth rates as compared to the vector control (Figure 1C), a possible effect of cell differentiation. When cultured in medium containing 1% serum, the difference in growth rate was even greater. At the low levels of serum, although some cells detached and died, extension of the neurite was greatly promoted (Figure 1C). Both the V1- and V2-transfected PC12 cells grew more slowly than the vector-transfected cells did. Examination of cell morphology indicated that the V1- and V2-transfected cells had a high proportion of cells undergoing modest autonomous differentiation with some neurite outgrowth compared to vector controls, when the cells were cultured in medium containing 15% serum, a serum condition that normally does not allow PC12 cell differentiation and neurite extension (Figure 1C). When the serum concentrations were reduced to 1% to facilitate cell differentiation and neurite growth, V1-transfected cells continued to grow and developed fully differentiated neuronal morphology with long processes, but the differentiation of the V2-transfected cells was not sustained and the neurites gradually disappeared, an indication of incomplete differentiation. Cell numbers were quantified by cell counting and the results were analyzed statistically. The experiments indicated that both the V1- and V2-transfected cells exhibited significantly reduced rates of proliferation in both 1% and 15% serum conditions as compared with the vector control (Figure 1D).

Versican affects nAChR subunit expression

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels that are expressed in PC12 cells [22]. The expression levels and profiles of nAChRs in the cells change dramatically along with morphological alterations in the process of neuronal differentiation [32]. NGF is a known potent factor in the induction of PC12 cell differentiation [21], effectively regulating nAChR expression in PC12 cells [33]. We have previously demonstrated that addition of NGF to the cultures had no additive effect on differentiation and neurite outgrowth of the V1-transfected cells [29]. To further study the role of V1 and V2 expression in neural differentiation, we confirmed that addition of NGF did not affect the morphology of these V1-induced, differentiated PC12 cells (Figure 2A). We then examined the expression profile of nAChR subunits by Western blotting, and investigated the difference in nAChR expression between versican transfection and NGF treatment (Figure 2B). The blotting bands on the films were semi-quantified using a densitometer, and the relative OD values of nAChR subunit expression in the V1-transfected cells with or without NGF treatments were summarized in Figure 2C. The results indicated that both V1 transfection and NGF treatment increased nAChR 7 subunit expression without affecting 2 subunit expression (Figure 2B), suggestive of a specific role for nAChR7 in PC12 cell neuronal differentiation. Interestingly, V1 and V2 transfection greatly enhanced nAChR 3 subunit expression but reduced nAChR 4 expression, while NGF treatment produced the opposite effects on nAChR 3 and nAChR 4 expression (Figures 2B and 2C).

Versican regulates nAChR function

We also measured nAChR-mediated current in PC12 cells by means of voltage-clamp recordings. In all V1 and V2-transfected cells, application of the AChR agonist carbachol (CCh, 250 μmol/l) evoked a fast inward current (Figure 3A, upper row and Figure 3B). Notably, the CCh-evoked current rapidly desensitized to a steady state (I_{SS}), which was characterized by a sharp on-set peak (I_P) (Figure 3A, upper row), yielding a smaller I_{SS}/I_P value (Figure 3C). In contrast, the majority of vector-transfected cells showed no response to CCh, while a few of them exhibited a small current. CCh also induced currents in NGF-treated PC12 cells (Figure 3A lower row). The CCh-currents in NGF-treated cells displayed a slower desensitization rate, thus a larger I_{SS}/I_P value (Figure 3C), in comparison to that of the versican-expressing cells. Treating the V1-transfected PC12 cells with NGF reduced the desensitization rate of the CCh-current (Figure 3C) without effect on the current amplitude (Figure 3B). These results further indicate that versican-induced neural differentiation occurs through a mechanism distinct from NGF signaling.

Silencing of V1 expression reverses its effects on cell morphological change and nAChR current.

To confirm the direct involvement of the versican V1 isoform in morphological and functional changes in PC12 cell differentiation,

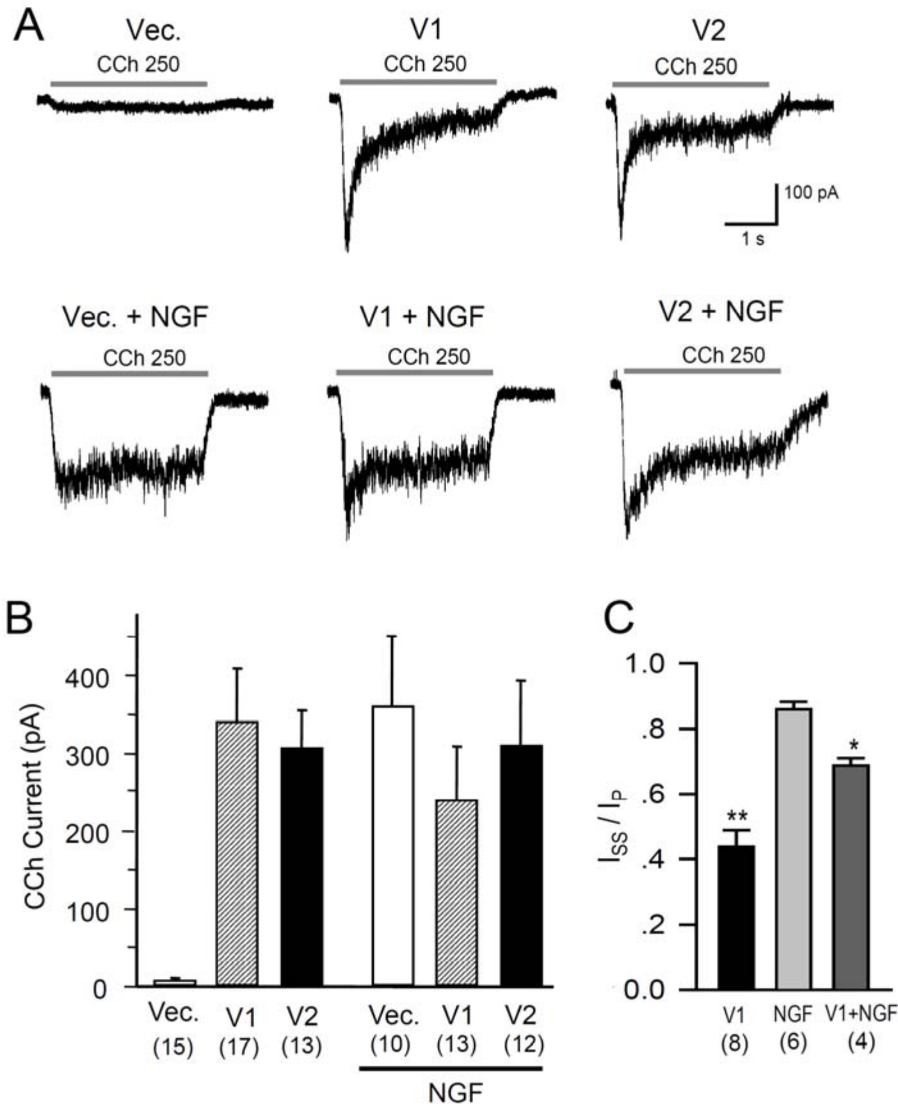

Figure 3. Versican alters the receptor-mediated current. A, Nicotinic currents in PC12 cells transfected with V1, V2 and a control vector in the presence or absence of NGF. In the absence of NGF, no currents were detected in the vector-transfected cells. In V1- or V2-transfected cells, a distinct nicotinic current was detected, which exhibited rapid activation and rapid desensitization, forming a sharp downward peak. In the presence of NGF, nicotinic currents were detected in all cell types, but the currents differed. Currents in V1- or V2-transfected cells sustained sharp peaks, but NGF-induced nicotinic currents in vector-transfected cells did not exhibit a sharp peak. The effects of versican V1 and V2 isoforms on nicotinic currents were quantified. Addition of NGF increased the currents of vector-transfected cells but had little effect on V1 and V2-transfected cells. B, The effects of NGF on nicotinic currents in the V1-, V2, and vector-transfected PC12 cells were analyzed in at least 10 cells, and the results were statistically analyzed using t-test. The numbers below the plotting bars indicate the number of the tested cell. C. Shown are the ratios of the steady state to the peak of CCh-evoked current in PC12 cells that were stably-transfected with versican V1 (0.44±.05), cells treated with NGF (0.86±0.2), and V1-expressing cells treated with NGF (0.69±0.2). The numbers below the plotting bars indicate the number of tested the cell. * $P < 0.05$; ** $P < 0.01$.

we used siRNA to reduce V1 expression in the V1-expressing PC12 cells. One construct (siRNA-ver10789), containing a target sequence against the G3 domain, was co-expressed with the G3 construct transiently in COS-7 cells to have a high efficiency for gene transfection and protein expression and was found to greatly silence expression of the G3

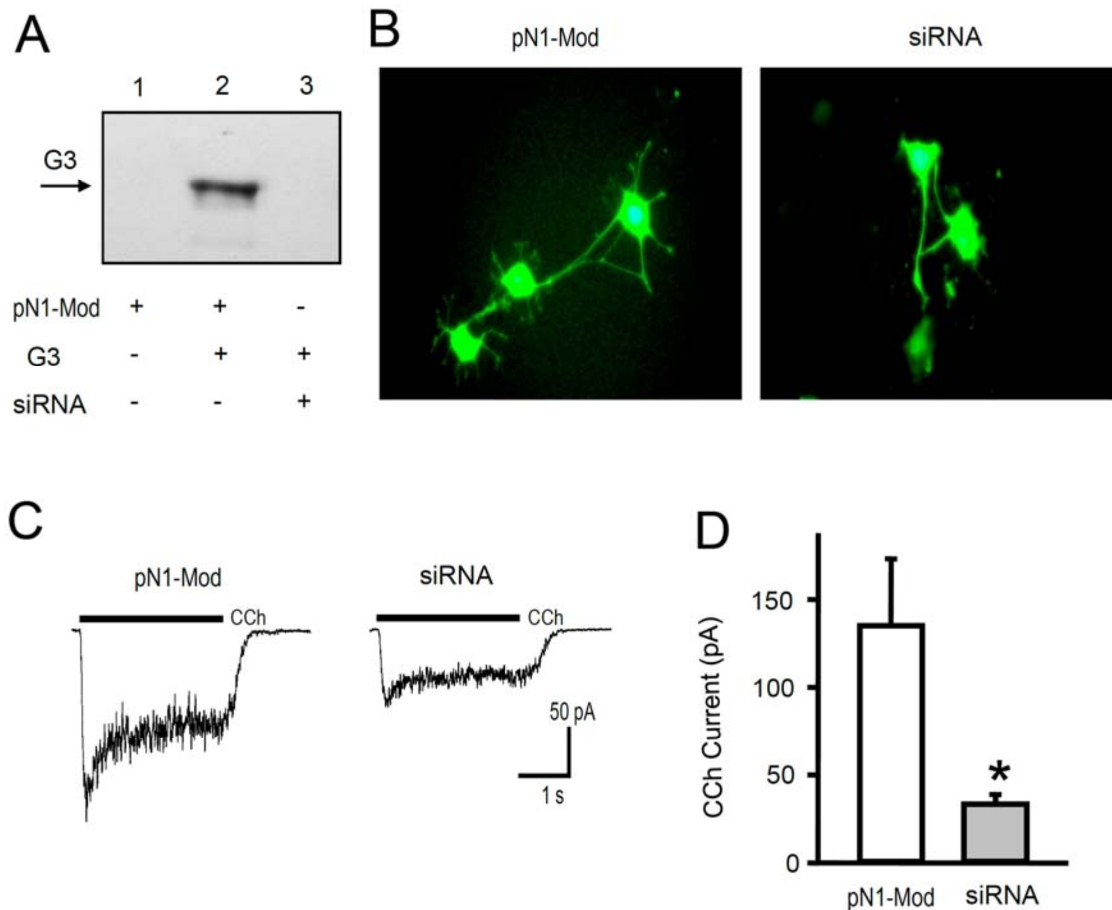

Figure 4. Reduction in V1 function using siRNA. A, COS-7 cells were co-transfected with the G3 construct and siRNA-ver10789 or the G3 construct and pN1-Mod. Cell lysate and culture medium were analyzed on Western blot probed with 4B6, and the same blot was re-probed with anti-actin antibody. Co-transfection with siRNA-ver10789 reduced G3 expression greatly. B, V1-expressing cells were transfected with V1-siRNA or pN1-Mod. Cell morphology was examined on fluorescent cells. Transfection with V1-siRNA reduced process extension. C, Nicotinic currents in the fluorescent PC12 cells were analyzed. Transfection with V1-siRNA exhibited lower levels of current activation, forming a small downward peak. D, The effects of V1-siRNA on nicotinic currents were analyzed with t-test statistically. V1-siRNA significantly reduced the nicotinic currents induced by versican ($n = 3$ * $p < 0.05$).

construct (**Figure 4A**). PC12 cells stably expressing versican V1 isoform were transiently transfected with the validated siRNA construct or a control vector pN1-Mod. The siRNA transfected cells were identified by the expression of green fluorescent protein that was tagged with the expression vector. Examination of cell morphology demonstrated that V1 silencing resulted in a reduction of cellular process extension (**Figure 4B**), confirming the key role for the versican V1 in process extension in non-neuronal cells [34], and neurite outgrowth in neuronal cells [29,30]. Analysis of nAChR-mediated currents in the fluorescent PC12 cells revealed that in comparison to the pN1-Mod controls, transfection of V1-siRNA reduced the amplitude of the CCh-evoked current (**Figure 4C**) with statistical significance (**Figure 4D**). We concluded that the changed phenotypes in the V1-transfected PC12 cells were due to increased versican expression, rather than a non-specific consequence of transfections.

Discussion

During development, the interactions between cells and the extracellular matrix (ECM) initiate

signals to regulate many fundamental processes. The ECM regulates cellular functions such as migration, adhesion, proliferation, differentiation, and morphogenesis, acting through a large diversity of receptors on the cell surface. The receptor-matrix interactions not only regulate gene activities but also cell behaviors. Due to the large proportion of ECM in the neural tissue mass, the extracellular environment may be particularly important in controlling neuronal differentiation and development. Considering that versican is one of the major extracellular proteoglycans in developing and mature brains, and that the expression levels of different versican isoforms change along with neuronal development, it is important to study the molecular mechanisms through which versican regulates neuronal differentiation, development and maturation.

The PC12 cell line was derived from rat pheochromocytoma, a tumor arising from chromaffin cells of the adrenal medulla. These cells have stem cell-like properties. They can: i) proliferate for self-renewal; ii) differentiate into neuronal-like cells [20]; and/or iii) undergo apoptosis [35]. This has been a useful model for studying neuronal differentiation and signaling [20]. NGF was the first, and to date, the strongest factor that induces PC12 cell differentiation. Our results demonstrated that versican is able to initiate neuronal differentiation in PC12 cells. However, only the V1 isoform could induce mature neuronal differentiation including fully developed neurites: In terms of neurite growth and nAChR-mediated currents, the scope of V1-induced PC12 cell differentiation was more extensive in comparison to NGF-induced differentiation. Previous studies have shown that different isoforms of versican exhibit distinct features of expression [8,9]. Versican V2 is present at relatively low levels during the late embryonic and early postnatal period, but increases dramatically during maturation. In contrast, the versican V1 levels double between E14 and birth, after which they decrease by more than 90% to reach a low "mature" level that remains stable throughout adulthood [8,36]. Thus, versican V2, along with brevican, becomes a major chondroitin sulfate proteoglycan in the mature brain [11]. These evident and clear-cut changes are highly suggestive of specific functions for versican V1 and V2 isoforms during neurogenesis and mature brain homeostasis, respectively. Our results that V1 actively induced neuronal differentiation and promoted neurite outgrowth, while V2 inhibited neurite outgrowth may be relevant to versican's expression patterns.

The nAChRs are protein complexes composed of five membrane-spanning subunits [37] that associate to form an ion-conducting pore through the plasma membrane. The nAChRs are widely expressed throughout the central and peripheral nervous system. Changes in the expression and function of nAChRs in the brain may be related to some neural disorders such as Alzheimer's disease [38]. Like other ligand-gated ion channels, the subunit composition of nAChR determines the channel's functions [39]. We found that versican V1- and V2-induced AChR currents were similar to the responses seen in stratum radiatum interneurons, with rapid onset and fast desensitization [40]. Although V1 and V2 exerted different effects in modulating PC12 cell differentiation and neurite outgrowth, their effects on nAChR currents and expression were similar. For example, both V1 and V2 down-regulated 4 of nAChR and up-regulated 3 expression, and it is known that 3 correlates with a fast onset current while 4 correlates with a slow rising current [40]. The process of desensitization has recently attracted increasing attention because of its capacity to influence synaptic transmission over extended periods, thus controlling the time course of synaptic events and enabling sustained changes in efficacy of synaptic transmission [41]. Versican greatly accelerated the process of nAChR desensitization, suggesting a role of ECM molecules in regulation of ion channel functions. In addition, since the kinetics of nAChR-mediated currents in versican expressing cells apparently differed that in NGF-treated PC12 cells, we propose that versican induces neuronal differentiation through a mechanism rather than NGF receptor activation.

Since each subunit of nAChR is unique, there exists substantial complexity in the structure, regulation, and function of neuronal nAChRs in PC12 cells [42]. The receptor of nACh exhibits distinct temporal and tissue specific patterns of expression in the peripheral nervous system, a source of PC12 cells, and central nervous system, and is Ca2+-permeable [43], which could affect a variety of cell activities

including neurite growth. Indeed, over-expression of nAChR 7 induces sustained Erk phosphorylation in PC12 cells [44], a pathway involved in neurite growth [29]. Nicotine can induce phosphorylation of Erk and CREB in PC12 cells [33]. We have previously demonstrated that expression of versican V1 isoform promoted phosphorylation of Erk and EGFR [29]. The above reports are in agreement with our results in the current study. Nevertheless, it is reported that addition of nicotine to culture medium decreases neurite outgrowth in PC12 cells in a concentration-dependent manner, which can be blocked by alpha-Bungarotoxin, an inhibitor of nAChR 7 [45]. The functional relationship between increased expression of nAChR 7 and exogenous addition of nicotine awaits further investigation.

Taken together, the successful cloning and expression of full-length versican V1 and V2 isoforms have allowed us to study their functions in defined conditions. Together with previous research, our results provide strong evidence that versican actively regulates neuronal differentiation and maturation, neurite outgrowth, and synaptic transmission. Further studies will shed light on the role of versican in nerve injury repair and the development of neuronal diseases.

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