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ORIGINAL PAPER

Neurite Outgrowth is Dependent on the Association of c-Src and Lipid Rafts

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Abstract Regulation of neurite outgrowth is an important aspect not only for proper development of the nervous system but also for tissue regeneration after nerve injury and the treatment of neuropathological conditions. Here, we report that neurite outgrowth in cortical neuron and neuro 2A (N2A) cell was dependent on intact lipid rafts, as well as the enhanced localization of c-Src in the lipid rafts. Src inhibition or lipid rafts disruption could specifically block c-Src phosphorylation profile, pY416 Src increase and pY529 Src decrease, they also resulted in pY529 Src and c-terminal Src kinase (Csk) partition out of lipid rafts. Thus, we concluded that c-Src signal cascades within the lipid rafts is crucial for efficient neurite outgrowth.

Keywords Neurite outgrowth, c-Src · Src phosphorylation · Lipid rafts

Abbreviation

MβCD	β-Cyclodextrin
MBS	MES buffered saline
B27	Serum-free medium supplement for neuron culture
PP2	4-Amino-5-(4-chlorophenyl)-7-(*t*-butyl) pyrazolo[3,4-d]pyrimidine

Introduction

Proper functioning of the nervous system requires connections between neurons and their targets. Undifferentiated cells have to expand cylindrical extensions with a growth cone at a distal tip in a process called neurite outgrowth [1]. Control over neurite outgrowth is fundamentally important in neuroscience implications, as well as for nerve regeneration [2]. Based on previous investigation, neurite outgrowth is regulated by a variety of signaling mechanisms, including growth factors and soluble or membrane bound guidance cues [3]. Some signals mediate initiation of neurite outgrowth at the cell soma, while others direct the growing neurite along a particular pathway by signaling at the growth cone [4]. Many of these signals ultimately converge upon specialized areas of the plasma membrane, lipid rafts [5]. There are studies showing the significant role of lipid rafts in the formation of neuronal polarity [6]. Phospholipids could regulate neurite outgrowth in cultured neurons and that the correct distribution of axonal membrane proteins requires the formation of this sphingomyelin/cholesterol rich microdomains [7]. Moreover, not only dendrite outgrowth but also axonal regeneration is dependent on intact lipid rafts [8, 9].

The findings also indicated that raft localized molecules were essential for neurite outgrowth. For example,

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increased level of c-Src is in part involved in axonal elongation [10, 11]. Lipid raft-localized c-Src is more catabolically active than non-raft localized one. When c-Src was recruited into lipid rafts, it will cluster and govern neurite inducing signals [12, 13].

Src activity was demonstrated to dependent to a large extent on the ability of lipid rafts to serve as docking platforms for extracellular ligands [14, 15]. It has become evident that Src protein is expressed at high levels in neurons and exists in a structurally distinct and activated form [16, 17]. Src kinase activity was related to tyrosine phosphorylation, which has been proposed to participate in neuritogenesis [18]. In addition, Src are enriched in nerve growth cones, where they interact with cytoskeleton and facilitate neurite elongation process [19, 20]. But the role of Src on neurite outgrowth was diverse in varied cell types [21–23]. Thereby, the present study employed cortical neuron and neuroblastoma N2A cell and was determined to underscore the important mechanism for distinct compartments as well as signal propagation related neurite outgrowth.

Methods

Chemicals

Dulbecco's modified Eagle's medium (DMEM) was purchased from Invitrogen (San Diego, CA), neuron-defined serum-free Neurobasal medium was from Gibco (Rockville, MD). Poly-L-lysine, MβCD and water-soluble cholesterol were from Sigma (St Louis, MO). Effectene transfection reagent was from Qiagen (Valencia, CA). Polyvinylidene difluoride membrane and ECF substrate were got from Amersham Biosciences (Pittsburg, PA). Antibodies, including c-Src, pY416Src and pY529Src were purchased from Upstate Biotechnology (Lake Placid, NY), flotillin-1 and Csk from Santa Cruz (Santa Cruz, CA). Alkaline phosphatase conjugated secondary antibody was from Novus Biological Inc. (Littleton, CO).

Neuronal Cell Culture and Treatment

Primary cultures of cortical neurons were from embryonic day 16 C57BL/6 mice. The protocol was approved by the Fudan University animal ethics committee. Animal treatment was followed guidelines set out in the *Guide for the Care and Use of Laboratory Animals* published by the National Institute of Health.

Cortices were dissected and collected as previously described [24]. Briefly, fetuses were decapitated and cortical tissue was collected under sterile conditions. After removing meninges, cortical tissue was dissociated in 0.05% trypsin at 37°C. Dissociated neurons were washed in DMEM and gently suspended in neuron-defined serum-free Neurobasal medium supplemented with B27.

N2A cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin. The cells were plated at 8.5×10^5/ml onto 100 mm culture dishes coated with 0.1% poly-L-lysine and incubated in 10% CO_2 at 37°C.

MβCD was reported to have capacity to delete membrane cholesterol. Cells were treated with MβCD (100 μM) in serum-free medium for 1 h at 37°C. To reconstitute cholesterol in the MβCD-treated cells, water-soluble cholesterol (1 mg/ml) in growth medium was added to the cell and incubated for additional 2 h at 37°C. For PP2, Src kinase inhibitor treatment, cells were exposed to 2 μM of PP2 for 1 h. The effect of subsequent wash out was also analyzed.

For the dominant-negative mutant of c-Src^{K295M} (DN Src) studies, cells were transfected with either DN Src or the corresponding control vector (PcDNA3) by using effectene transfection reagent. Twelve to sixteen hours after transfection, cells were rinsed with standard growth medium and incubated for additional 24 h prior to analysis.

Neurite Outgrowth Assay

For neurite estimation, neurites including branches were manually traced by the investigator under the phage-contrast microscopy. Neurite-bearing cells were counted in populations of 200–300 cells from four or five randomly chosen fields. Neurites were defined as processes longer than one and a half cell diameters [25].

Detergent-Free Preparation of Lipid Rafts and Immunoblotting

The isolation of lipid rafts in the current study was adapted from Lisanti's lab [26, 27]. Cells were scraped into 2 ml of 500 mM sodium carbonate, PH11.0. Homogenization was carried out sequentially in the following order using a loose-fitting Dounce homogenizer (10 strokes), three 10 s bursts of a Polytron tissue grinder (Brinkmann Instruments, Inc., Westbury, NY) at setting 6, followed by one 30 s burst at setting 4 and one 30 s burst at setting 8 of a sonicator equipped with a micro-probe (Heat systems-Ultrasonics, Inc., Plainview, NY). The homogenate was then adjusted to 45% sucrose by the addition of 2 ml of 90% sucrose prepared in MBS at pH 6.8 and placed at the bottom of an ultracentrifuge tube. The lysate was then overlaid with 4 ml of 35% sucrose and 4 ml of 5% sucrose, both prepared in MBS containing 250 mM sodium carbonate at pH 11. The discontinuous gradient was

centrifuged at 39,000 rpm for 16–20 h in a SW41 rotor. A light-scattering band to the 5–35% and 35–45% sucrose interface was collected and the total proteins were separated on a 10% SDS-polyacrylamide gel, then transferred to a polyvinylidene difluoride membrane. Western Blot analyses were carried out with antibodies for various c-Src kinase, Csk and flotillin 1, respectively. Primary antibodies were probed with alkaline phosphatase conjugated secondary antibody (1:5000). Protein bands were detected by the addition of the ECF substrate, and the intensities were quantified and analyzed using ImageQuant software.

Confocal Microscopy

Cortical neurons and N_2A cells were grown on glass coverslips that were acid-cleaned and pretreated with 0.1% poly-lysine until 50% confluent. The cells were fixed with 3.7% paraformaldehyde in PBS (pH7.4) for 30 min at room temperature and blocked with PBS diluent (0.3%Triton X-100, 1% normal donkey serum, 1% bovine serum albumin, and 0.01% sodium azide, pH7.2). Then the cells were subsequently incubated with anti-flotillin-1 (1:200) and Alexa 488-conjugated secondary antibody for 1 h at room temperature. After wash, the cells were further incubated with anti-Src and Alexa 594-conjugated secondary antibody for 1 h at room temperature. Finally, the coverslips were dipped in distilled water and mounted by NO-FADE (PBS: glycerol 9:1, 0.1% *p*-phenylenediamine, pH8.0). Confocal fluorescence microscopy was carried out using Bio-Rad MRC 1024 with 63× oil objective.

Data Analysis

All quantitative data were expressed as mean ± SEM and analyzed with Prism 5 software. For all data sets, normality assumptions were reached, validating the application of the one-way ANOVA, followed by *t*-test for multiple comparisons. Differences were considered significant for $P < 0.05$.

Results

Neurite Outgrowth was Dependent on Intact Lipid Rafts

The integration of insights from both primary culture systems and clonal cell lines is quite essential for fully understanding of general mechanisms of neuronal behavior. In the present experiment, cortical neurons were mainly used for examining axonal neurite outgrowth, while N_2A cells for dendrite analysis.

As demonstrated in Fig. 1, cortical neurons were grown for 10 days, neurite-bearing cells were 90.5 ± 5.8% of culture cells. In N_2A cells with 70–80% confluence, neurite bearing cells were 91.1 ± 6.2% of culture cells. MβCD and water-soluble cholesterol were generally used to disrupt or restore lipid rafts respectively. We observed that neurite-bearing cells were 32.6 ± 2.3% cortical neurons and 30.5 ± 1.8% of N_2A cells under MβCD treatment. Water-soluble cholesterol resulted in the restoration of neurite-bearing cells, the number was 85.1 ± 3.5% and 89.6 ± 2.0% of culture cells in cortical neurons and N_2A cells, respectively.

Modulation of Lipid Rafts in Neural Cells

Sucrose gradient centrifugation was well established for lipid rafts analysis. After 16 h of centrifugation, two bands appeared at the interface between 5–35% and 35–45% sucrose gradient not only in cortical neurons but also in N_2A cells. The band at the interface between 5 and 35% was displayed high flotillin-1 immuno-reactive signal, was thereby considered as lipid rafts microdomain in neural cells. In contrast, the band at the 35–45% sucrose interface represented the non-raft fraction. Flotillin-1 immuno-positive signals were expressed by the ratio of densities in lipid rafts to two fractions. It was demonstrated that flotillin-1 expression was 0.91 ± 0.09 in cortical neuron and 0.92 ± 0.06 in N_2A cells. MβCD and water-soluble cholesterol exposure led to changes in flotillin-1 distribution. The relative densities are 0.54 ± 0.13 and 0.87 ± 0.11 in cortical neuron, 0.49 ± 0.09 and 0.89 ± 0.07 in N_2A cells (Fig. 2).

Effect of c-Src on Neurite Outgrowth

Src kinase was revealed to be involved in lipid rafts modulation by many previous studies [21, 22]. It was also associated with neurite outgrowth [28]. In the current experiment, we demonstrated in Fig. 3 that neurite-bearing cells was deceased to 35.3 ± 2.2% and 34.1 ± 1.9% of cortical neurons and N_2A cells respectively by 2 μM PP_2 exposure. They were 89.0 ± 5.1% and 90.9 ± 4.8% of culture cells when PP2 wash out. Concurrently treatment with PP_2 and MβCD, neurite-bearing cells were 34.5 ± 4.3% and 33.9 ± 3.7% of cortical neurons and N_2A cells respectively, which had not significant difference to their treatment individually. c-Src could be specifically downregulated by the c-Src^{K295M} (DN Src) input, the expression level was decreased 67%. It was shown that neurite-bearing cells were 30.2 ± 2.5% and 34.5 ± 3.0% cortical neurons and N_2A cells by DN Src transfection, which had statistical significance compared with the corresponding vector (Mock, 89.2 ± 5.9% and 91.0 ± 6.1%) treatment. MβCD had not additive effect either (data not shown).

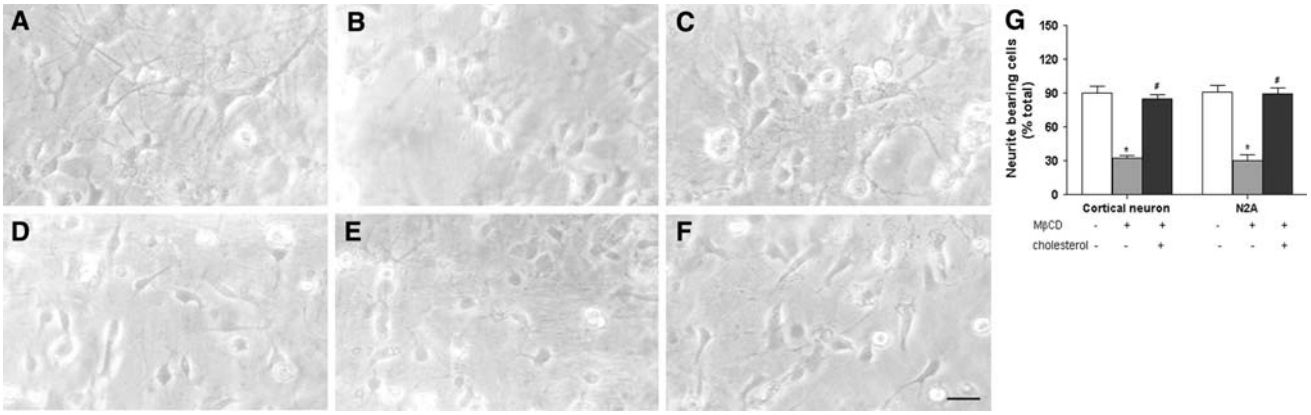

Fig. 1 Neurite outgrowth was dependent on intact lipid rafts**.** Cortical neurons were grown for 10 days (**a**, **b**, **c**) and N2A cells for 70–80% confluence (**d**, **e**, **f**), then the cells were exposed to vehicle (**a**, d), 100 μM of MβCD (**b**, **e**) and 1 mg/ml of water-soluble cholesterol (**c**, **f**) for the indicated time. Fibrous elements were resembled neurite extensions, the neurite bearing cells were quantified and expressed as percentage of total cells. Each value represents mean ± S.D. of five independent experiments performed in triplicate (G). $P < 0.05$ * *vs* control, # *vs* MβCD treatment. Scale bar, 10 μm

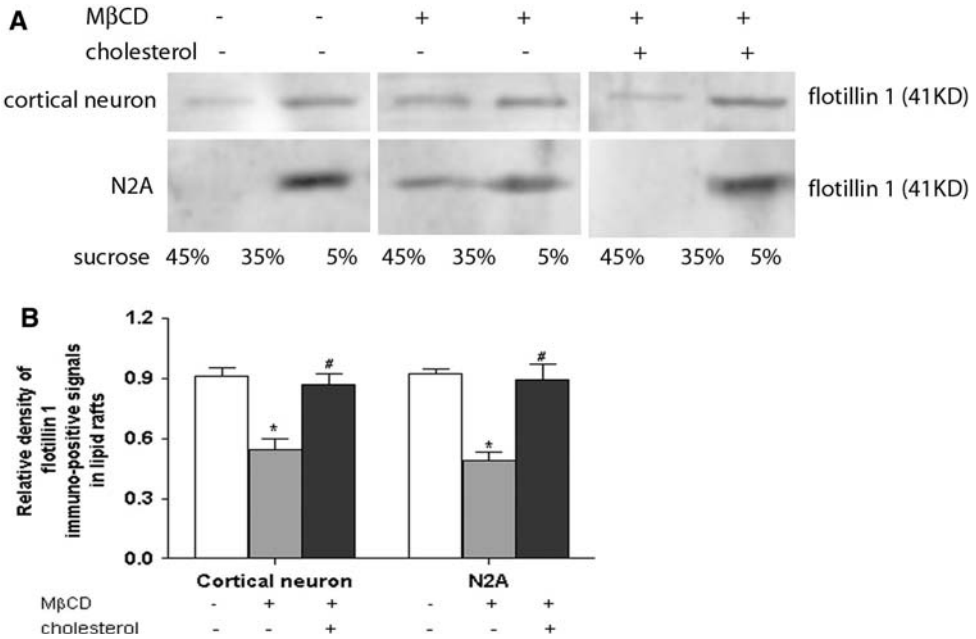

Fig. 2 Modulation of lipid rafts integrity in Neural cells**.** Cells were exposed to vehicle, 100 μM of MβCD and 1 mg/ml of water-soluble cholesterol for the indicated time. Then the lipid rafts/flotillin-1 enriched membrane fractions were purified by discontinuous sucrose gradients centrifugation as described in material and methods. The proteins from light-scattering bands to the 5–35% and 35–45% sucrose interface were separated with SDS-PAGE and the presence of flotillin-1 antibody (**a**). The data was calculated as the ratio of relative densities in lipid rafts to total, and each value represents mean ± S.D. of five independent experiments performed in triplicate. $P < 0.05$ * *vs* control, # *vs* MβCD treatment (**b**)

Association of c-Src and Lipid Rafts Integrity

As shown in Fig. 4, c-Src immuno-positive signals were mainly distributed within flotillin-1 enriched fraction in cortical neurons and N_2A cells. Under confocal microscopy, c-Src immuno-positive signals were almost co-localized with that of flotillin-1, they displayed with yellow color (A). The result was confirmed by Western blot analysis. Immuno-positive signals for Flotillin-1 and c-Src were mainly distributed in 5–35% sucrose interface in both cortical neuron and N2A cells (B). When transfected with DN Src, flotillin-1 enriched fraction was disappeared, the relative densities for flotillin-1 immuno-positive signals in lipid rafts were decreased from 0.87 ± 0.18 to 0.45 ± 0.17 in

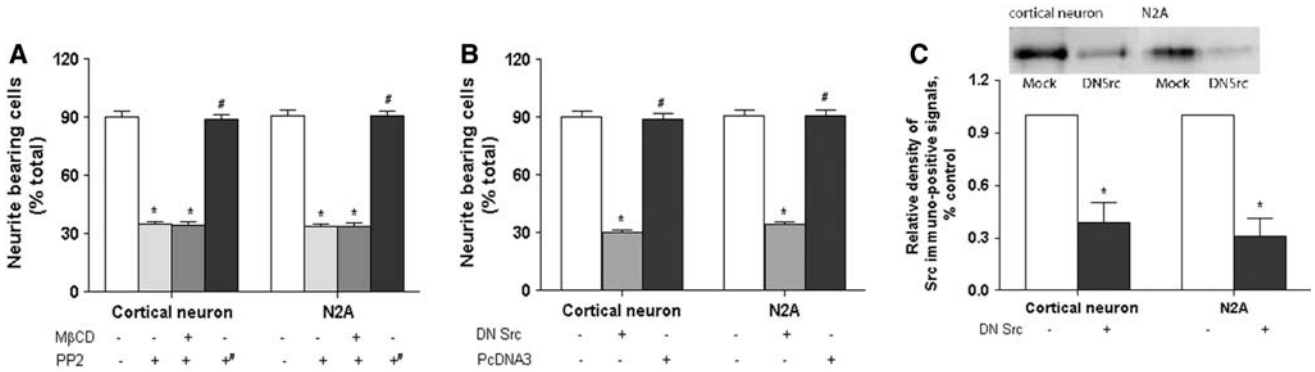

Fig. 3 Effect of c-Src on neurite outgrowth**.** Neural cells were exposed to vehicle, 100 μM of MβCD, and 2 μM of PP2 for the indicated time (**a**). Or the cells were transfected with either a plasmid coding for dominant-negative mutant of c-Src^{K295M} (DN Src, 0.5 μg) or the empty expression vector (PcDNA3, Mock) (**b**), neurite outgrowth was quantified and expressed as percentage of total culture cells. After transfection, Src kinase expression was analyzed by Western Blot (**c**), data was expressed by the relative densities of control (Mock). Each value represents mean ± S.D. of five independent experiments performed in triplicate. $P < 0.05$, * *vs* control, # *vs* PP2 or DNSrc transfection

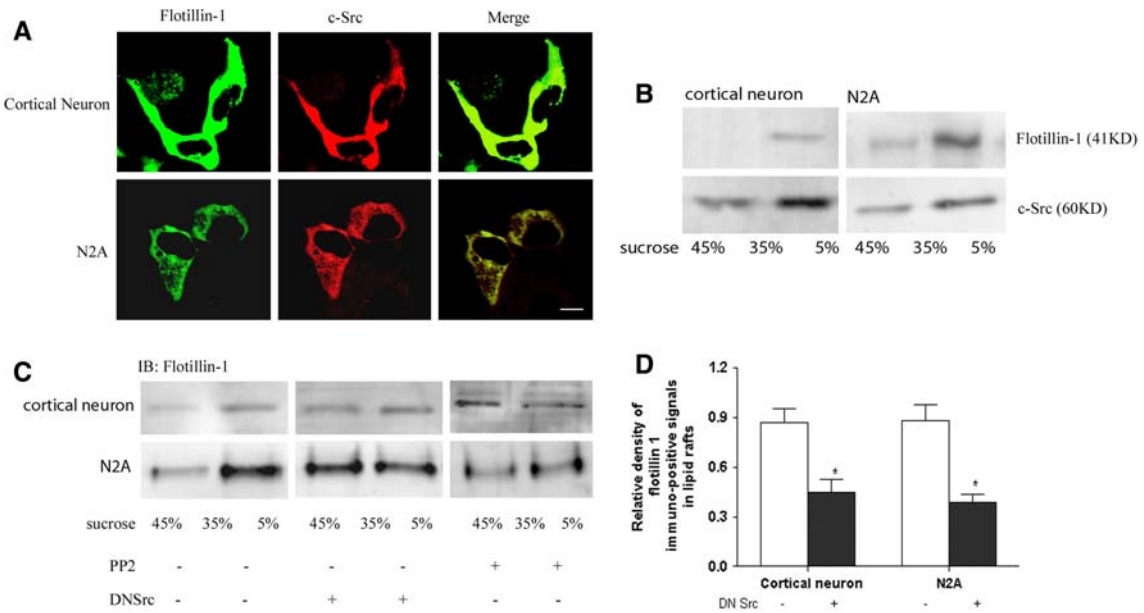

Fig. 4 Association of c-Src and flotillin-1 in neural cells**.** Neural cells were grown for the indicated time, then fixed with paraformaldehyde, permeabilized with Triton X-100, and stained with anti-flotillin-1 and Alexa 488-conjugated secondary antibody, anti-Src and Alexa 594-conjugated secondary antibody subsequently. Flotillin-1 immuno-positive signals were stained by green, and c-Src kinase by red. Colocalized components were shown by yellow. Confocal fluorescence microscopy was carried out using Bio-Rad MRC 1024 and 60 × objective, Scale bar, 10 μm (**a**). Neural cells were centrifuged in discontinuous sucrose gradients, proteins from light-scattering bands to the 5–35% and 35–45% sucrose interface were separated with SDS-PAGE and the presence of flotillin-1 and c-Src antibodies (**b**). Cells were exposed to 2 μM of PP2, or transfected with either a plasmid coding for dominant-negative mutant of c-Src (DN Src, 0.5 μg) or the empty expression vector (PcDNA3), then the flotillin-1 enriched membrane fraction was purified by discontinuous sucrose gradients (**c**), flotillin-1 expression in 5–35% sucrose interface was calculated as the relative densities to total. Each value represents mean ± S.D. of three independent experiments. * $P < 0.05$ (**d**)

cortical neurons and 0.88 ± 0.21 to 0.39 ± 0.11 in N_2A cells (C and D). Similar results were also revealed by PP2 treatment, flotillin-1 immuno-positive signals in lipid rafts were decreased from 0.85 ± 0.21 to 0.41 ± 0.11 in cortical neurons and 0.86 ± 0.16 to 0.40 ± 0.19 in N_2A cells (C).

c-Src Phosphorylation was Dependent on Intact Lipid Rafts

pY416 and pY529 were characterized as two residues that are responsible for the c-Src kinase architecture and

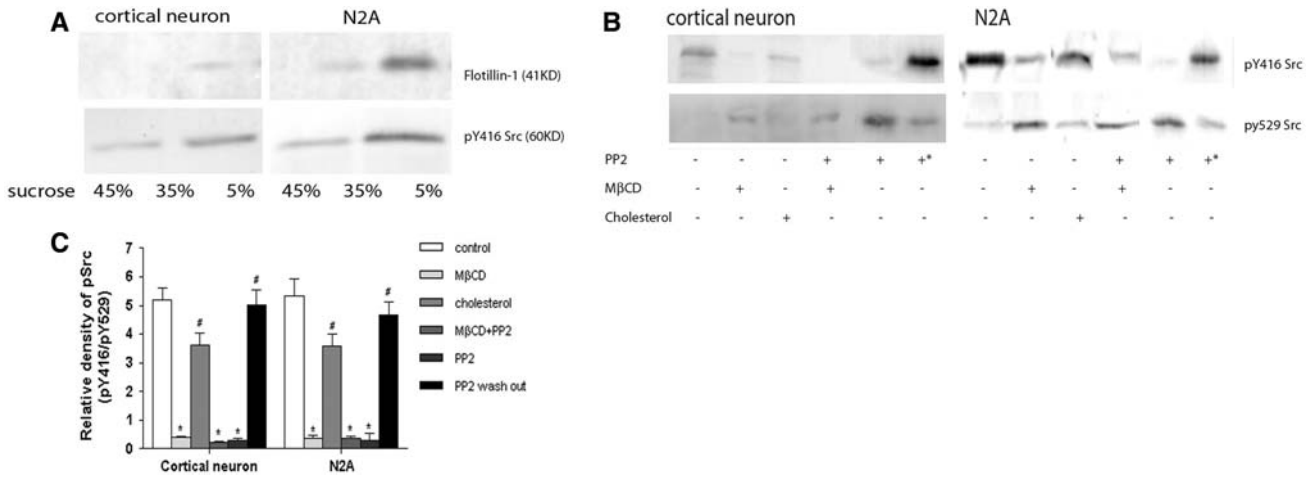

Fig. 5 c-Src phosphorylation required intact lipid rafts. Neural cells were grown for the indicated time, flotillin-1-enriched membrane fractions were purified by sucrose density gradients centrifugation. The proteins from light-scattering bands to the 5–35% and 35–45% sucrose interface were separated with SDS-PAGE and probed by pY416Src (**a**). Cells were exposed to vehicle, 100 μM of MβCD, 1 mg/ml of water-soluble cholesterol, and 2 μM of PP2 for the indicated time. Proteins from the whole cell lysis were subjected to SDS-PAGE and the presence of pY416 Src and pY529Src (**b**), data was calculated as density ratio of pY416 to pY529, each value represents mean ± S.D. of three independent experiments. $P < 0.05$, * *vs* control, # *vs* MβCD, MβCD + PP2 or PP2 (**c**)

activity. In the present study, pY416Src immuno-positive signals were observed to be concentrated in flotillin-1 enriched fraction (Fig. 5a). In contrast, immuno-positive signals for pY529Src were very weak in the basal condition.

Strikingly, immuno-positive signals for pY416Src and pY529Src in the cell extract could be affected by lipid rafts disruption or c-Src inhibition. As shown in Fig. 5b, the ratio of expression densities for pY416Src to pY529Src was 5.21 ± 0.91 in cortical neurons and 5.34 ± 1.32 in N_2A cells with vehicle treatment (Fig. 5b).

c-Src phosphorylation could be affected by lipid rafts modulation. After MβCD exposure, the expression ratio of pY416Src to pY529Src were changed accordingly, it was decreased from 0.40 ± 0.10 in cortical neurons. In N_2A cells, it was 0.36 ± 0.26. Water-soluble cholesterol could partly restore the effect of MβCD, the expression levels of pY416Src/pY529Src were 3.62 ± 0.95 in cortical neurons, 3.58 ± 0.92 in N_2A cells. pY416Src and pY529Src expression could also be influenced by c-Src modulation. PP2 treatment resulted in ablated pY416Src and enhanced pY529Src expression, the density ratio were 0.31 ± 0.10 and 0.29 ± 0.14 in cortical neuron and N_2A cells, respectively. The effect was restored by PP2 wash out, the expression ratio for these two phosphorylation profiles were 5.01 ± 1.17 in cortical neurons, 4.67 ± 1.03 in N_2A cells. Additionally, MβCD and PP2 concurrently treatment also resulted in the decrease in pY416/pY529, but with no significant difference with their individual treatment. Data illustrated as 0.23 ± 0.08 in cortical neurons, 0.36 ± 0.15 in N_2A cells (Fig. 5c).

Association of pY529Src and Csk with Lipid Rafts

We hypothesized that pY529Src and Csk send off their duty for efficient neurite outgrowth regulation by shuttling in or out of lipid rafts. When the distribution was expressed by the ratio of lipid rafts or non-raft to total densities, pY529Src expression were 0.49 ± 0.12 and 0.51 ± 0.13 in cortical neuron, 0.43 ± 0.11 and 0.57 ± 0.13 in N_2A cells in control group, they changed to 0.14 ± 0.05 and 0.86 ± 0.15, 0.15 ± 0.04 and 0.85 ± 0.12, respectively with PP2 exposure. DNSrc transfection also resulted in the alteration of pY529Src distribution. The relative densities of pY529Src in raft and non-raft fractions were 0.53 ± 0.14 and 0.47 ± 0.13 in cortical neuron, 0.55 ± 0.13 and 0.45 ± 0.11 in N_2A cells, which displayed significant difference with corresponding vector transfection (0.14 ± 0.02 and 0.86 ± 0.16 in cortical neuron, 0.11 ± 0.02 and 0.89 ± 0.14 in N2A cells).

Csk distribution was also detected here. Data demonstrated that Csk expression was 0.92 ± 0.22 and 0.08 ± 0.01 in raft and non-raft fractions of cortical neurons, 0.95 ± 0.20 and 0.05 ± 0.01 in N2A cells in control group. PP2 exposure led to similar change pattern to that happened on pY529Src distribution. The relative densities in raft and non-raft fractions were 0.21 ± 0.09 and 0.79 ± 0.32 in cortical neuron, 0.25 ± 0.06 and 0.75 ± 0.14 in N_2A cells. In cortical neuron, DNSrc transfection could dramatically change Csk distribution. The relative densities were 0.21 ± 0.11 and 0.79 ± 0.24 in lipid raft and non-raft fractions, while they were 0.94 ± 0.23 and 0.06 ± 0.02 when transfected with corresponding vector. In N_2A cells, the relative densities for

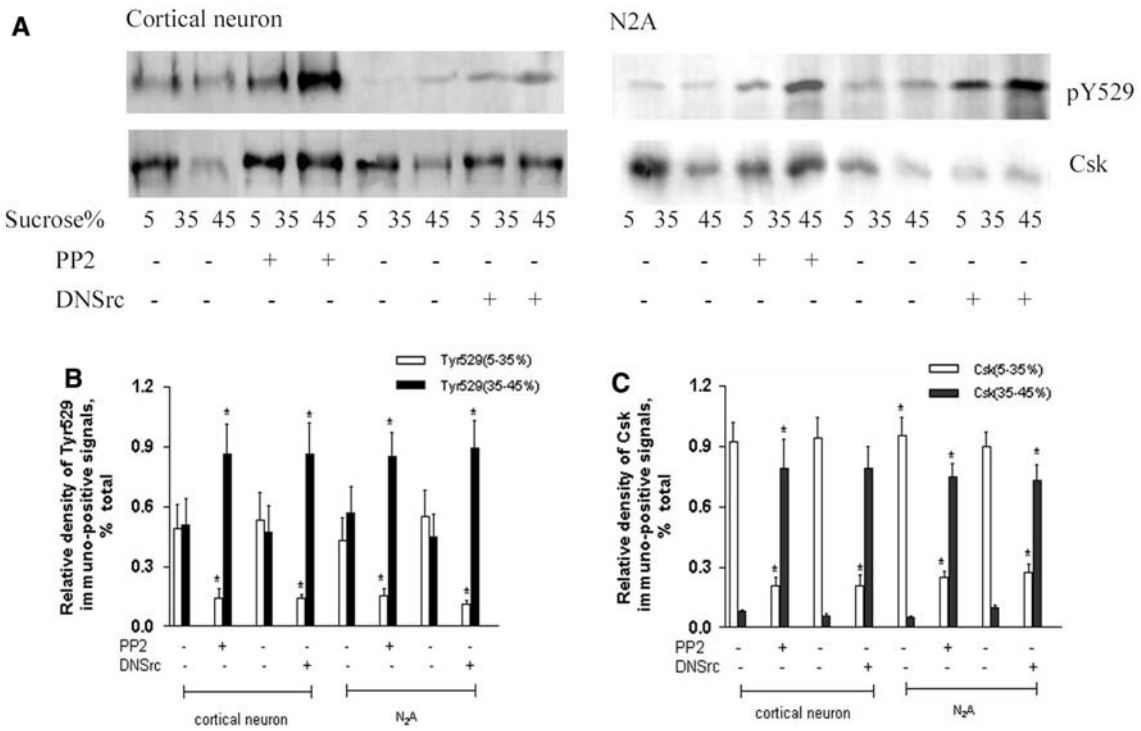

Fig. 6 pY529Src and Csk were excluded from lipid rafts. Neural cells were grown for the indicated time, then exposed to 2 μM of PP2 or transfected with DNSrc. flotillin 1-enriched membrane fractions were purified by discontinuous sucrose gradients centrifugation. Proteins from light-scattering bands to the 5–35% and 35–45% sucrose interface were separated with SDS-PAGE and the presence of pY529Src and Csk antibodies (**a**). Data was expressed as relative densities to total, each value represents mean ± S.D. of three independent experiments. * $P < 0.05$ (**b**, **c**)

Csk were 0.90 ± 0.15 and 0.10 ± 0.02 in control group, 0.27 ± 0.10 and 0.73 ± 0.17 in DNSrc transfection group (Fig. 6).

Discussion

Regulation of neurite outgrowth is an important aspect not only for proper development of the nervous system but also for tissue regeneration after nerve injury. It is well recognized in PC12 cells that neurite protrusion might be determined by the presence of specific areas of the plasma membrane, lipid rafts [29]. This is also the case for cortical neuron and N2A cells.

Now we demonstrated, in agreement with the reports on PC12 cells, that axonal outgrowth in cortical neurons and dendritic outgrowth in N2A cells were required intact lipid rafts. When the lipid rafts was reconstituted by water-soluble cholesterol, neurite outgrowth was invincibly recovered.

As we know, lipid rafts are the specialized membrane composition, which is enriched in cholesterol and sphingolipids [30, 31]. Many studies indicated that lipid rafts may act as platforms for concentration of signaling molecules important for cell adhesion, axon guidance and synaptic transmission in neuronal cells [32]. Membrane ruffle usually resulted in pronounced changes in molecule translocation and neurite extensions. When the signal molecules confined to lipid rafts, they will temporally and spatially facilitate the precise wiring of complex neuronal connections [33].

Flotillins are neuron specific lipid raft proteins, whose upregulation occurred in regenerating retinal ganglion axons and co-localizing with growth cones [34, 35]. In the present study, translocation of flotillin-1 immuno-positive signals could be provoked by approaches targeting lipid rafts disruption or c-Src inhibition. c-Src immuno-positive signals was usually distributed within flotillin-1 enriched fraction, this partition was also altered by c-Src blockade or lipid rafts disruption. It is now widely accepted that membrane microdomain lipid rafts was closely associated with c-Src kinase, c-Src could induce redistribution of flotillins from the plasma membrane to late endosomes and lysosomes [36]. Thus, the present data provided further evidence that conferred to the association of c-Src and lipid rafts, as well as the involvement of protein kinase in the neuronal functions.

As reported, c-Src activity is strictly regulated by a conformational mechanism that is controlled by

phosphorylation [37]. Two tyrosine residues, one is pY416 located in the kinase domain is required for its full activity, the other is pY529, which is located in the C-terminal region leading to the kinase inactivation [38]. The phosphorylation of pY529 is catalyzed by another tyrosine kinase, known as Csk [39, 40]. Clearly, the switch between the inactive and active states involves the interactions with SH3 domain for the stimulatory effect and the intramolecular interactions between SH2 domain and the COOH-terminal tail for the inhibitory effect. Besides that, the biological activity of c-Src requires membrane association [7, 41]. At the cell surface, Src may be segregated or anchored by glycophosphatidylinositol (GPI) and thereby modulate various signalling and membrane trafficking events.

It is displayed in the present study that pY416Src and pY416Src expression in the whole cell extracts were decreased and increased, respectively in response to lipid rafts disruption or Src kinase blockade. The later also triggered the partition of pY416Src and C-terminal Src kinase (Csk) out of lipid rafts. Combined with finding that dynamic phosphotyrosine signals are directly correlate with neurite outgrowth. Src dependent signals were distributed within the growth cone [9, 13], we can assume that compartmentation of c-Src within the lipid rafts was necessary for its active state, it can then recruit and phosphorylate lipid rafts related adaptor molecules and finally trigger neurite outgrowth signaling processes. When lipid rafts integrity was disrupted, restrained form of c-Src will be increased by the enhanced capability to bind Csk and the partition of pY416Src and Csk out of lipid rafts, which resulted in the disorder of neurite outgrowth modulation. Strikingly, this finding may contribute to the understanding that neuron development and regeneration specifically rely on the intact lipid rafts and their organized components.

Conclusions

We demonstrated that enhanced c-Src activity within the lipid rafts was essential for neurite outgrowth modulation. Compartmentation of c-Src signaling cascades within the lipid rafts was prerequisite for its recruitment and phosphorylating lipid rafts related adaptor molecules, and finally trigger neurite outgrowth signaling processes.

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