RESEARCH ARTICLE

# p75 and neural cell adhesion molecule 1 can identify pathologic Schwann cells in peripheral neuropathies

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## Abstract

**Objective**: Myelinated Schwann cells (SCs) in adult peripheral nerves dedifferentiate into immature cells in demyelinating neuropathies and Wallerian degeneration. This plastic SC change is actively involved in the myelin destruction and clearance as demyelinating SCs (DSCs). In inherited demyelinating neuropathy, pathologically differentiated and dysmyelinated SCs constitute the main nerve pathology. **Methods**: We investigated whether this SC plastic status in human neuropathic nerves could be determined by patient sera to develop disease-relevant serum biomarkers. Based on proteomics analysis of the secreted exosomes from immature SCs, we traced p75 neurotrophin receptor (p75) and neural cell adhesion molecule 1 (NCAM) in the sera of patients with peripheral neuropathy. **Results**: Enzyme-linked immunosorbent assay (ELISA) revealed that p75 and NCAM were subtype-specifically expressed in the sera of patients with peripheral neuropathy. In conjunction with these ELISA data, pathological analyses of animal models and human specimens suggested that the presence of DSCs in inflammatory neuropathy and of supernumerary nonmyelinating or dysmyelinating SCs in inherited neuropathy could potentially be distinguished by comparing the expression profiles of p75 and NCAM. **Interpretation**: This study indicates that the identification of disease-specific pathological SC stages might be a valuable tool for differential diagnosis of peripheral neuropathies.

# Introduction

Inflammatory peripheral neuropathy encompasses various types of neuropathies associated with nerve inflammation, including Guillain–Barré syndrome (GBS) and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP).[1] GBS can be further classified into acute inflammatory demyelinating polyradiculoneuropathy (AIDP), acute motor axonal neuropathy (AMAN) and Miller Fisher syndrome (MFS), and the generation of anti-ganglioside antibodies is commonly related to the development of AMAN and MFS.[2] The discovery of anti-ganglioside antibodies also provides important insight into the early diagnosis of AMAN or MFS. In contrast, even though autoantibodies against nodal/paranodal proteins, such as neurofascin or contactin, have been found in a few cases of CIDP,[1,3] how AIDP and CIDP develop is unclear. No pathomechanism-relevant serum biomarkers of these demyelinating neuropathies have been developed to date. In addition, CIDP is frequently confused with Charcot–Marie–Tooth disease type 1a (CMT1a), the most common inherited demyelinating neuropathy, because of similarities in their clinical courses and electrophysiological findings.[4]

Recent studies have demonstrated that the phenotypic changes of Schwann cells (SCs), the peripheral glia, such as dedifferentiation, are implicated in the pathophysiology of demyelinating neuropathies.[5,6] SC dedifferentiation indicates a phenotypic transition of mature SCs into an immature type and is typically found during Wallerian degeneration (WD) after axonal injury.[5,6] Dedifferentiated SCs activate several self-myelinolytic mechanisms, including autophagolysosomes, to contribute to myelin clearance during WD.[7,8] Interestingly, dedifferentiated SCs also appear to be actively involved in inflammatory demyelination as components of "demyelinating SCs (DSCs)".[6] However, abnormal differentiation of immature SCs results in supernumerary nonmyelinating or dysmyelinating SCs in CMT1a, thereby contributing to the development of demyelinating neuropathy.[9,10] Although these pathologically differentiated SCs might not be absolutely comparable to DSCs, several pathological similarities in SC phenotypes between inherited and traumatic demyelination have been observed.[11,12]

To provide insight into the development of specific serum biomarkers to represent pathological SCs in peripheral demyelinating neuropathy, in this study, we profiled exosomes of cultured primary SCs using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and then traced potential biomarker targets in the sera and sural nerves of peripheral neuropathy patients. The present study provides a novel potential tool for the identification of disease-specific SCs by enzyme-linked immunosorbent assays (ELISAs) performed on human serum.

# Materials and Methods

## Subjects

Serum samples were collected from 36 CIDP (10 females, 26 males), 14 AIDP (three females, 11 males), 20 AMAN (seven females, 13 males), and 39 CMT1a (17 females, 21 males) patients, as well as 20 healthy controls (14 females, six males). The diagnoses of CIDP, GBS (AIDP, AMAN), and CMT1a were made according to the respective clinical and laboratory diagnostic criteria.[13–15] We first classified GBS subtypes as AIDP and AMAN based on electrophysiological findings.[16] To further clarify the classification, only the patients with positive IgG anti-GM1 antibody from AMAN were included in the AMAN subgroup from this study. We used the patient serum of typical CIDP type, but not of multifocal acquired demyelinating sensory and motor neuropathy or distal acquired demyelinating symmetric polyneuropathy. Samples were then centrifuged at 1612 *g* for 10 min to separate the serum. Anti-ganglioside antibody assays were performed with ELISAs as described previously.[17] The research protocol was approved by the institutional review board of Dong-A University (No. HR-004-02), Dong-A University Hospital (Nos. 13-042) and Samsung Medical Center (No. 2017-11-152) in Korea. Measurements of serum p75 and neural cell adhesion molecule 1 (NCAM) were performed with commercially available ELISA kits (R&D Systems, Minneapolis, MN). All tests for p75 and NCAM were repeated three times in triplicate according to the manufacturer's instructions.

## Animals

All surgical procedures were performed according to protocols approved by the Dong-A University Committee on animal research, which follow the guidelines for animal experiments that were established by the Korean Academy of Medical Sciences (No. DIACUC-16-21). For a sciatic nerve injury, left sciatic nerves of adult C57BL/6 mice were axotomized as previously described.[5] Lewis rats for experimental allergic neuritis (EAN) were purchased from the Jackson Laboratory, EAN was generated by injection of the P2 peptide and the clinical stages were evaluated as previously described.[18] Peripheral myelin protein 22 (PMP22) transgenic mice (C22)[19] were obtained from Samsung Medical Center (Seoul, Korea). The mouse model contains seven copies of the human PMP22 gene, which leads to a demyelinating neuropathy.

## Primary SC culture and exosome purification

Primary SC culture from neonatal rat sciatic nerves was performed as previously reported.[20] For biochemistry and collection of conditioned medium, cells were used at 2–4 generations. SCs were cultured with DMEM containing 5 μmol/L forskolin, 30 ng/mL neuregulin-1 and 1% exosome-free FBS (obtained by serum ultracentrifugation at 100,000*g* for 12 h). Exosomes were isolated by ultracentrifugation-based isolation techniques.[21]

## Protein identification

The tryptic peptides derived from SC exosomes were analyzed by reverse-phase nano-LC-MS/MS using an Easy nLC 1000 system coupled online to an LTQ Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The resultant list of identified proteins was compared to the exosome database ExoCarta (www.exocarta.org), and gene ontology was analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID, v6.8).

## Histology and western blot analysis

The cryosections of sciatic nerves and paraffin sections of human sural nerves were immunostained with anti-NCAM, anti-myelin basic protein and anti-p75 antibodies. Alexa Fluor 488 or Cy3-conjugated antibodies were used for the secondary antibodies. Immunofluorescence staining and western blot analysis were performed as previously described.[20] The sources of antibodies and reagents will be provided upon request.

## Statistical analyses

Receiver operating characteristic (ROC) curves were analyzed to estimate the diagnostic performance of the respective tests between each group using MedCalc version 18.11.3 (MedCalc Software bvba, Ostend, Belgium). The cutoff points for the calculation of sensitivity and specificity were determined by ROC curves. ELISA data were analyzed using one-way analysis of variance followed by a Kruskal–Wallis test and Sidak's multiple comparisons test using GraphPad Prism version 6.01 (GraphPad Software Inc., La Jolla, CA). For statistical analysis of western blot results and of the number of immunoreactive cells in sections, unpaired Student's *t*-tests were performed. The results are expressed as the mean ± standard error of the mean. A value of $P < 0.05$ was considered significant.

# Results

## Identification of NCAM and p75 from the sera of peripheral neuropathy patients

To develop potential serum biomarkers for pathologic immature SCs in human neuropathic nerves, we first identified secreted exosomal proteins from cultured immature SCs by proteomic analysis (Fig. 1A). Exosomal peptide-containing fractions were analyzed by nLC-MS/MS, and the raw MS data were processed using the Proteome Discover[TM] software. In total, 13,116 peptides corresponding to 1356 protein groups were identified in the rat SC exosomes (false discovery rate <1%). The list of the top 100 proteins can be found in Table S1. Out of the 1356 proteins, 696 had been catalogued as rat-derived exosomal proteins in the ExoCarta database (Fig. 1B). Notably, the rat SC-derived exosomal proteins included p75 and NCAM, well-known immature SC proteins, and western blotting confirmed a significant enrichment of p75 and NCAM in the rat SC-derived exosomes (Fig. 1C).

We next examined p75 and NCAM levels in the sera of patients with various peripheral neuropathies using ELISAs (Fig. 1D and E). We found threefold higher levels of serum p75 in CIDP (256 ± 31.26 pg/mL, $P < 0.01$) and AIDP (207.3 ± 37.85 pg/mL, $P < 0.01$) patients than healthy controls (73.69 ± 40.58 pg/mL). Serum NCAM levels in CIDP (4960 ± 476 pg/mL, $P < 0.01$) and AIDP (4729 ± 661 pg/mL, $P < 0.01$) patients were also higher than those of healthy controls (2298 ± 303 pg/mL). However, the serum levels of NCAM and p75 in the AMAN group were not significantly different from the control levels, although they did tend to be higher than those of the controls. Interestingly, the highest levels of serum NCAM were observed in the sera of CMT1a patients (6663 ± 277 pg/mL), while serum p75 levels of CMT1a patients (12.74 ± 5.315 pg/mL) were similar to those of the controls.

From the ROC curves, we determined the optimal cutoff values for p75 and NCAM according to each group. In the case of p75, the CIDP group, which showed the highest level, had a 92.1% sensitivity and 95.0% specificity compared to the CMT1a group at 56.45 pg/mL (AUC = 0.986, $P < 0.001$) (Fig. 1F). CIDP was distinguished from AMAN at 103.40 pg/mL (AUC = 0.782, 63.2% sensitivity and 85.0% specificity, $P < 0.001$) but not from AIDP (AUC = 0.596, 84.6% sensitivity and 40.0% specificity, $P = 0.365$), which also showed high levels of p75. In the case of NCAM, the CMT1a group, which showed the highest levels, was revealed to have a clear difference from CIDP at 7038.69 pg/mL (AUC = 0.672, 39.5% sensitivity and
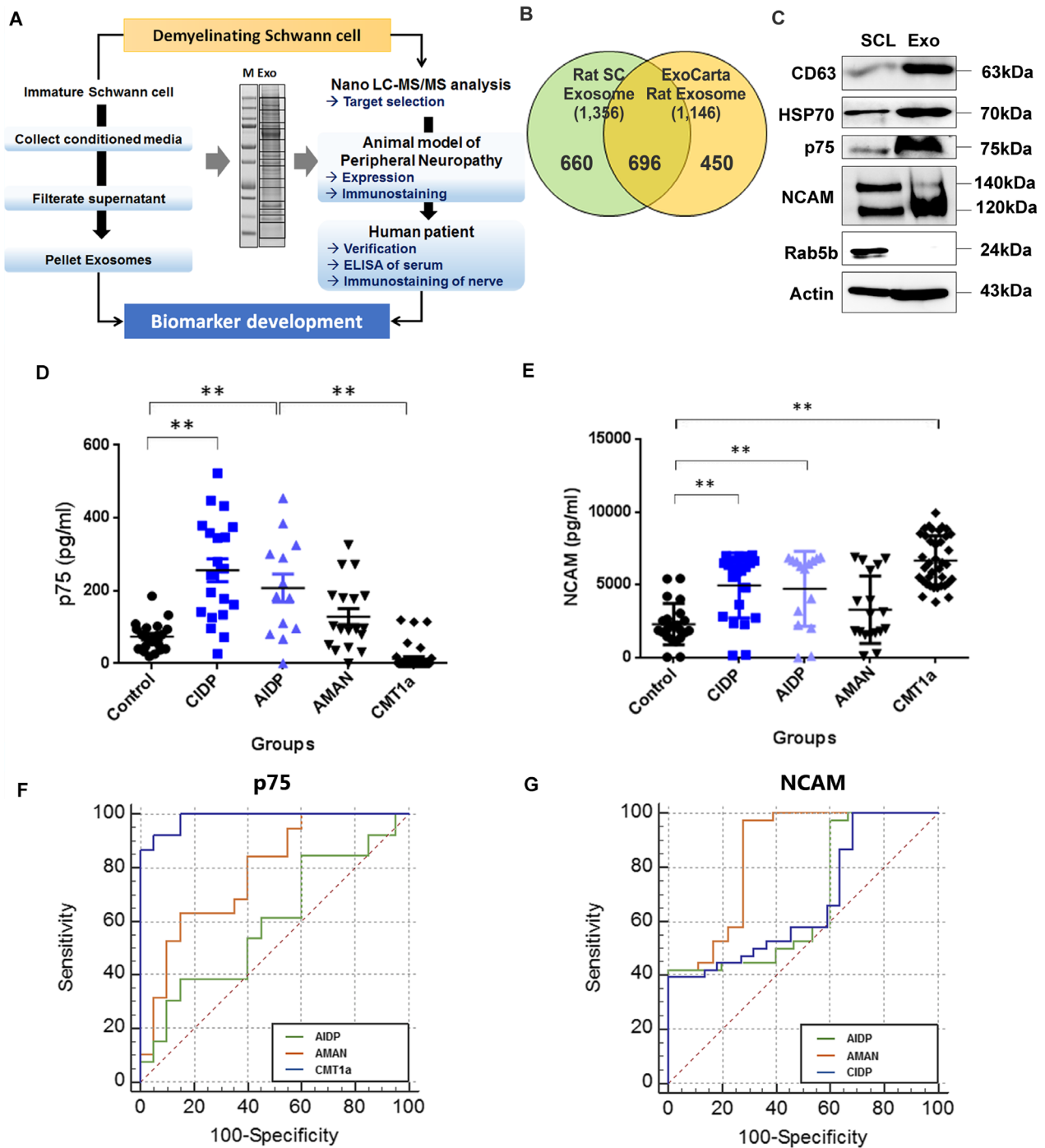
**Figure 1.** Schwann cell exosomal p75 and NCAM were reflected in the human sera of peripheral neuropathy patients. (A) Workflow for the development of a biomarker in demyelinating neuropathy. (B) Venn diagram depicting the overlap of the exosomal proteome obtained in the present study (rat Schwann cell [SC] exosomes) and the ExoCarta database (ExoCarta rat exosomes). The numbers represent the total numbers of proteins identified in this study and the rat exosomes studied to date. (C) Characterization of SC exosomes (Exo) by western blot analysis. SCL; Schwann cell lysate, Rab5b; a cytosolic protein marker. (D and E) Detection of serum p75 and NCAM in peripheral neuropathy patients by ELISAs. Significant differences between patient groups and healthy controls by ANOVA are denoted by **$P < 0.01$. (F and G) ROC curves for the detection of p75 (F) and NCAM (G) by ELISAs. NCAM, neural cell adhesion molecule 1; ROC, receiver operating characteristic; ELISAs, Enzyme-linked immunosorbent assays.

100% specificity, $P = 0.017$), AIDP at 6896.14 pg/mL (AUC = 0.679, 42.1% sensitivity and 100% specificity, $P = 0.033$), and AMAN at 3937.07 pg/mL (AUC = 0.852, 97.4% sensitivity and 72.2% specificity, $P < 0.001$) (Fig. 1G). Taken together, they suggest that measuring NCAM and p75 levels in the sera of human neuropathy patients might be a valuable tool for differentiating inflammatory and inherited demyelination.

## Differential expression profiles of NCAM and p75 in pathological immature SCs in human neuropathic nerves

To determine whether the differential induction of p75 and NCAM in patient sera specifically represents differential expression levels of these proteins in pathologically immature SCs in human neuropathic nerves, we next examined the expression profiles of p75 and NCAM in the sural nerves of neuropathy patients. In the sural

nerves of CIDP patients, we found that ~25% of myelinating SCs showed perimyelin p75 expression in the abaxonal cytoplasm (Fig. 2A and D). In contrast, perimyelin p75 expression was barely observed in the sural nerves of CMT1a patients (Fig. 2A and D). Additionally, a comparison of the intensity values of p75 immunofluorescence showed a significant difference between CIDP and CMT1a groups (Fig. 2E), a correlative result of serum levels of p75 in both patient groups.

In the sural nerve of CIDP patients, NCAM staining was observed in both nonmyelinating SCs (Fig. 2B) and myelinating SCs (Fig. 2B–E). Consistent with our ELISA data showing the highest serum levels of NCAM in the CMT1a group among the neuropathy patients, the expression of NCAM in the CMT1a sural nerves was much higher than that in CIDP patients (Fig. 2B and C), and the expression was observed not only in supernumerary nonmyelinating SCs, including onion bulb (OB) cells, but also in dysmyelinating SCs (Fig. 2B and C).
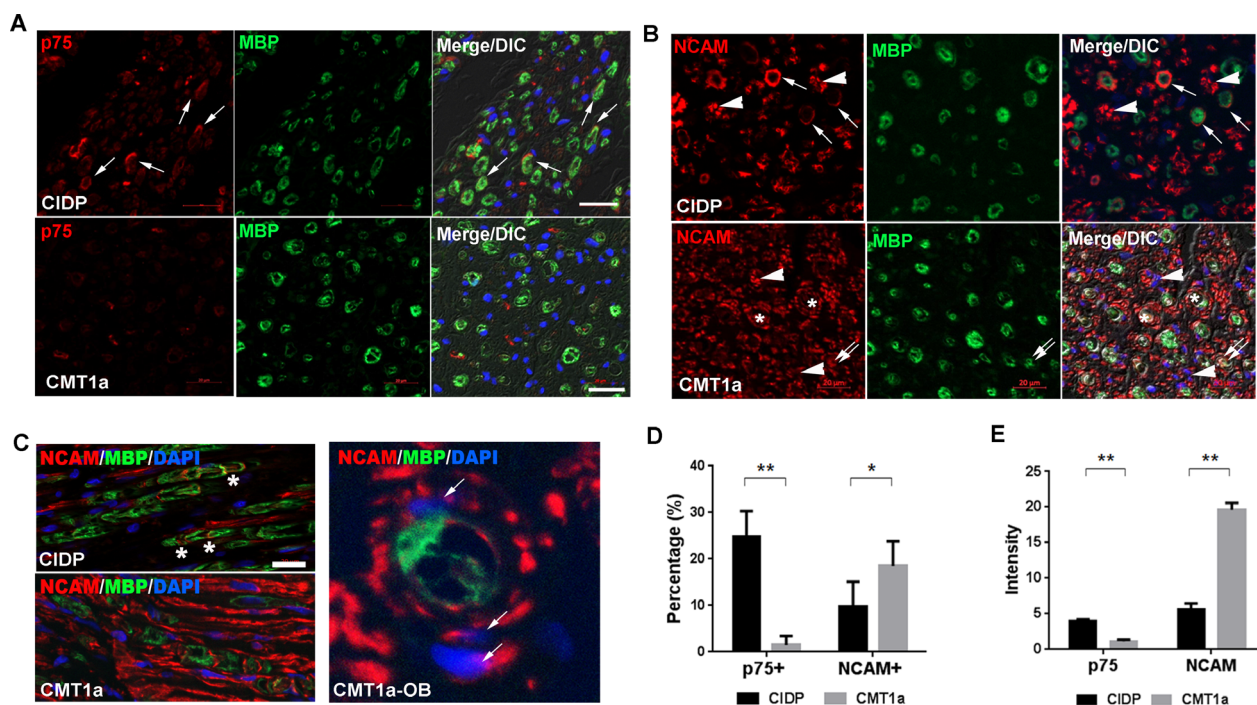


**Figure 2.** Differential p75 and NCAM expression in SCs of human peripheral neuropathies. (A and B) The expression profiles of p75 (A) and NCAM (B) in the sural nerves of human CIDP and CMT1a patients. Arrows; demyelinating SCs. Arrowheads; NCAM in nonmyelinating SCs. Asterisk; supernumerary onion bulb (OB) cells (enlarged in Fig. 2C). Double arrows; dysmyelinating SCs. Scale bar, 20 μm. (C) The expression profiles of NCAM in the sural nerves of human CIDP and CMT1a patients. NCAM staining of longitudinal sections (left two panels) showed differential levels of NCAM expression in CIDP and CMT1a sural nerves. MBP, myelin basic protein. Asterisk: Schmidt–Lanterman incisure. Scale bar, 20 μm. The right panel: the enlarged image of an OB in CMT1a. Arrows: nuclei. All the concentric cytoplasmic layers of the OB SCs expressed NCAM. (D) The graph shows the percentage of p75- or NCAM-expressing DSCs from 600 to 1800 MBP-positive SCs in sural nerve sections of CIDP (p75: 24 ± 1.975; NCAM: 9.84 ± 2.155) and CMT1a patients (p75: 1.5 ± 0.957; NCAM: 18.5 ± 2.661). Unpaired Student's *t*-test; *$P < 0.05$, **$P < 0.01$. (E) Fluorescence intensity values of p75 and NCAM staining in sural nerve biopsies. Unpaired Student's *t*-test; **$P < 0.01$. NCAM, neural cell adhesion molecule 1; CIDP, chronic inflammatory demyelinating polyradiculoneuropathy; CMT1a, Charcot–Marie–Tooth disease type 1a; SCs, Schwann cells.

## Acute primary and secondary demyelination and remyelination did not induce NCAM expression in myelinating SCs

Our findings indicate that NCAM in myelinating SCs was induced in chronic demyelinating conditions. To determine whether acute axonal degeneration-induced demyelination or acute inflammatory demyelination involves NCAM expression in DSCs, we examined the expression profiles of SC NCAM and p75 in the animal models of WD and acute inflammatory demyelinating neuropathy. In accordance with previous results,[22] p75 expression, which is normally expressed only in non-myelinating SCs in uncut control nerves, was highly induced in almost every DSC at 6 days after axotomy (Fig. 3A–C). In contrast, an axotomy did not induce the expression of NCAM in DSCs, and NCAM expression was not significantly increased in remyelinating SCs after crush injury (Fig. S1).

In the sciatic nerves of EAN, the expression of p75, but not NCAM, was also dramatically induced in DSCs during the whole course of the disease (Fig. 3D and E). Interestingly, nonmyelinating SCs exhibited a significant increase in NCAM expression in EAN animals (Fig. 3D). Western blot analysis also showed the induction of both p75 and NCAM in the sciatic nerve extracts (Fig. 3E and F). These findings indicated that the phenotypes of most DSCs in acute inflammatory demyelination would be p75+/NCAM-, which is similar to the DSCs in WD, and that NCAM expression in nonmyelinating SCs may contribute to the high levels of serum NCAM in AIDP patients.

## Differential mechanisms underlying the high serum NCAM levels in inflammatory versus inherited demyelination

The high expression of NCAM in nonmyelinating SCs in EAN and DSCs in the CIDP led us to examine whether NCAM expression was inducible by inflammatory cytokines in cultured primary SCs. Western blot analysis revealed that interferon gamma, tumor necrosis factor-alpha, and the nitric oxide donor sodium nitroprusside significantly induced NCAM expression (Fig. 3G). However, neuregulin, transforming growth factor-beta, and endoplasmic reticulum stress inducers did not upregulate NCAM expression in cultured primary SCs. In contrast to the expression of NCAM, p75 expression in SCs was not induced by any proinflammatory cytokines we tested (Fig. 3G).

Since the nerves in CMT1a are not inflammatory, the increased numbers of pathologically differentiated SCs, called supernumerary SCs,[9] might be related to serum NCAM elevation in CMT1a neuropathy. To confirm this hypothesis, we employed C22 mice, a mouse model of *Pmp22*-overexpressing CMT1a.[19] In accordance with previous results,[19] the sciatic nerves were poorly myelinated in C22 mice at postnatal weeks 5 and 8 (Fig. 3H). At postnatal week 8, NCAM immunoreactive cells were more frequently found in the sciatic nerves of C22 mice than control nerves (Fig. 3I and J). Indeed, the number of DAPI-positive cells was approximately twofold higher in C22 sciatic nerves (786 $\pm$ 73, $P < 0.01$) than wild-type (WT) nerves (322 $\pm$ 26), but the macrophage infiltration was minor (45.7 $\pm$ 5.6 [C22] vs. 26.3 $\pm$ 11.7 [WT], $P < 0.01$). The high level of NCAM expression in C22 nerves was also confirmed by western blot analysis (Fig. 3K).

## Discussion

In the present study, we tried to identify disease-specific pathological SC from human neuropathy patient serum and found that inflammatory DSCs, which are dedifferentiated after normal differentiation and myelination, and dysmyelinating or supernumerary immature SCs, which might never be fully differentiated, could potentially be distinguished by the differential expression profiles of NCAM and p75 not only in the tissues but also in the patient sera (Fig. 4). In accordance with previous results,[23] the present study showed that the induction of p75 in myelinating SCs is specific to demyelination either by axonal degeneration or inflammation. Unexpectedly, we could not detect the induction of NCAM in DSCs after axotomy and rarely observed it in the myelinating SCs of EAN neuropathic nerves. Although NCAM induction has been reported in the distal stump of the sciatic nerves after injury[24] and is known to be regulated by axon-SC contacts as p75,[25] the NCAM induction in the DSCs after nerve injury has not been clearly determined. In an immunoEM study, NCAM induction after injury was mainly localized in nonmyelinating SCs.[26] In addition, NCAM mRNA expression in the distal stump was not increased within 1 week after axotomy,[27] and Thornton et al.,[28] showed that NCAM induction in the distal stump of injured nerves derived from regenerating axons but not from denervated SC. Finally, even though Roche et al., reported the potential induction of NCAM in chronic axonal neuropathy, the cellular identity of NCAM induction in myelinating or nonmyelinating SCs was not determined.[29] Therefore, considering our immunostaining and western blot data, we think that the induction of p75, but not NCAM, appears to be specifically related to the immature cell transition of fully differentiated myelinating SCs to DSCs under acute demyelinating conditions or axon degeneration, and this could be reflected in the
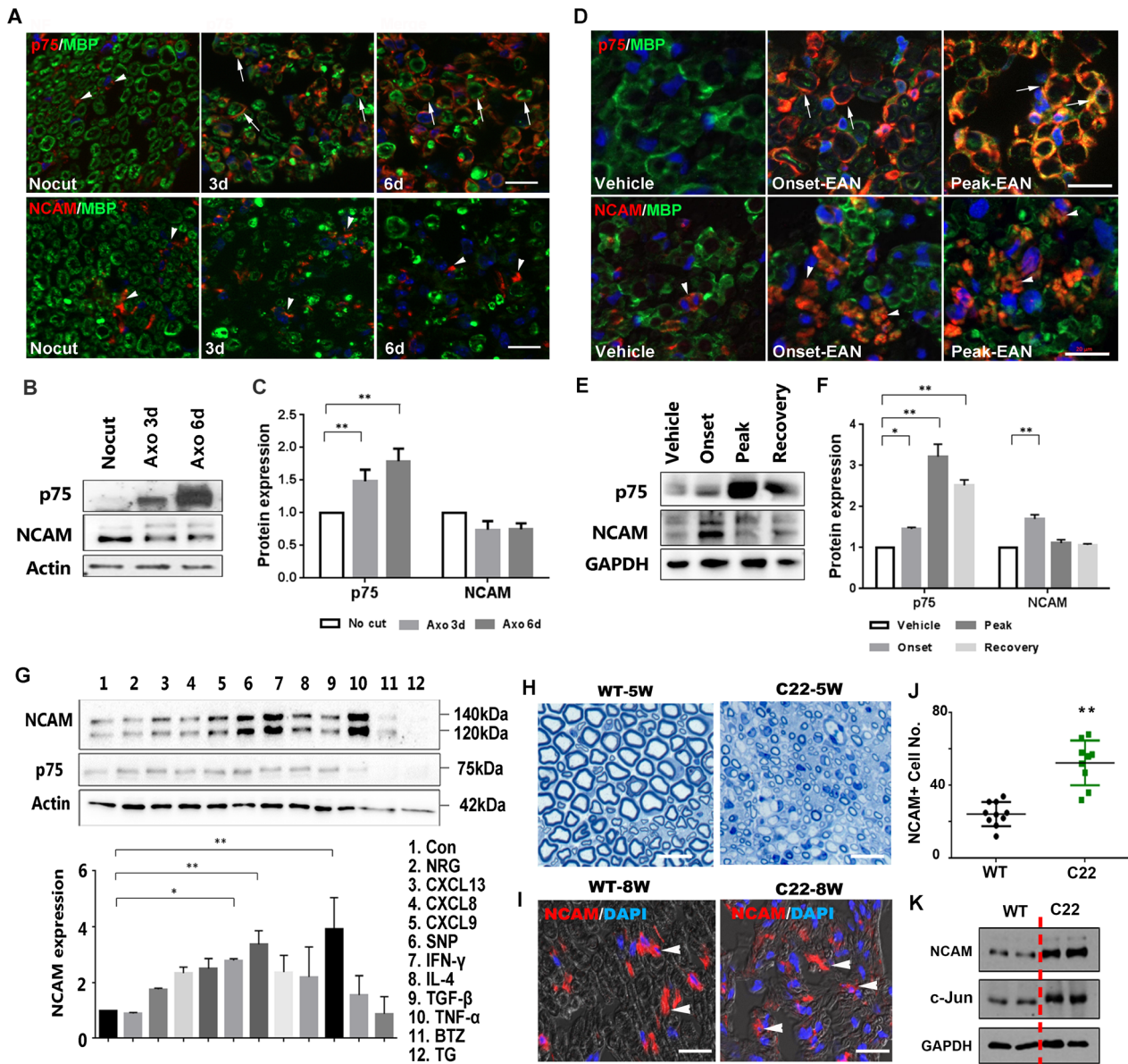
**Figure 3.** Differential p75 and NCAM expression in SCs in animal models of demyelination. (A) Axotomy induced the expression of p75, but not NCAM, in demyelinating SCs (DSC, arrows). Only nonmyelinating SCs (arrowheads) showed NCAM expression before and after axotomy. Scale bar, 20 μm. (B and C) Western blot analysis showed that the expression of p75, but not NCAM, was induced in the sciatic nerves after axotomy (Axo). The graph (C) shows quantitative values of p75 and NCAM expression levels (mean ± SEM, unpaired Student's *t*-test from three independent experiments; **$P < 0.01$). (D) DSCs at the onset and peak phases of EAN showed p75 induction (arrows), but NCAM staining was increased in nonmyelinating SCs (arrowheads). (E and F) Western blot analysis showed that p75 and NCAM were induced in the sciatic nerves during the course of EAN. The graph (F) shows quantitative values of p75 and NCAM expression levels (mean unpaired Student's *t*-test from three independent experiments; *$P < 0.05$, **$P < 0.01$). (G) The effects of 1 day treatment of cytokines and growth factors on the expression of NCAM and p75 in cultured primary SCs. NRG, neuregulin; BTZ, bortezomib; TG, thapsigargin; SNP, sodium nitroprusside. The graph shows the quantitative values of NCAM expression levels in three independent experiments. Mean ± SEM, unpaired Student's t-test; *, $P < 0.05$, **, $P < 0.01$. (H) Myelination profiles of the sciatic nerves of wild-type (WT) and C22 mice. Semithin plastic sections showed significant hypomyelination in C22 mice; 5W, postnatal 5 weeks. Scale bar, 20 μm. (I) Expression profile of NCAM in the sciatic nerves of WT and C22 mice at postnatal week 8 (8W). Arrowheads: nonmyelinating SCs. Scale bar, 20 μm. (J) The numbers of NCAM-positive cells in the sciatic nerves of WT and C22 adult mice. A dot indicates the number in a unit area (200x200 μm) within the sciatic nerve section. Unpaired Student's t-test; **, $P < 0.01$. (K) The increased expression of NCAM in C22 nerves was confirmed by western blot analysis. The expression of c-jun was used for a marker for immature SCs. NCAM, neural cell adhesion molecule 1; EAN, experimental allergic neuritis; SCs, Schwann cells; SEM, standard error of the mean.
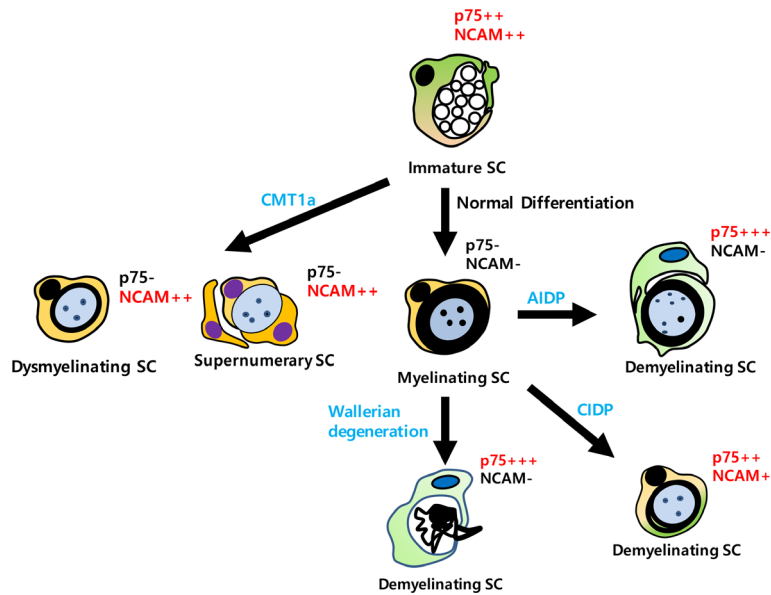
**Figure 4.** Pathological immature SC model in peripheral neuropathies. Differential characteristics of various types of demyelinating SC based on p75 and NCAM were displayed. After normal differentiation, myelinating SCs lose the expression of p75 and NCAM, both of them are expressed in immature SCs. Axonal injury or acute inflammatory condition lead myelinating SCs to dedifferentiate into p75+/NCAM- demyelinating SC which is not identical to developing immature SCs. In chronic inflammation, myelinating SCs acquires the expression of both p75 and NCAM. In CMT1a nerves, persistent NCAM expression was observed in abnormally differentiated SCs. In this figure, the potential phenotype changes of nonmyelinating SCs at adulthoods were not included. NCAM, neural cell adhesion molecule 1; CMT1a, Charcot–Marie–Tooth disease type 1a; SCs, Schwann cells.

patient serum. In addition, this result further supports the idea that the dedifferentiated myelinating SCs are not exactly identical to developing immature SCs.[6]

Serum NCAM levels were recently shown to be high in peripheral demyelinating neuropathies.[30] However, this study did not explain the origin and pathologic implications of NCAM in demyelinating neuropathy. These findings suggested that NCAM could have a neuronal origin based on a previous report showing the expression of NCAM in demyelinating axons in the central nervous system.[30–32] We also found a high serum level of NCAM in AIDP and CIDP patients, but pathological analyses revealed that inflammatory conditions increased the expression of NCAM in SCs. The induction of NCAM by proinflammatory cytokines in cultured SCs further supports this finding. Thus, our results are consistent with those of a previous report[30] and provide a potential SC origin of high serum NCAM in inflammatory demyelinating neuropathies.

On the other hand, we found a high level of NCAM, but not p75, in the serum from CMT1a patients, and this finding is consistent with previous[33,34] and the current histological data showing NCAM+/p75- supernumerary nonmyelinating SCs, including OB SCs, and dysmyelinating SCs in the sural nerves of CMT1a patients. Extensive evidence for abnormal differentiation of SCs as an

underlying pathomechanism of CMT1a has been provided,[23,34,35] and our findings indicate that the pathological immature SC status in CMT1a is not the dedifferentiated status or demyelinating status of fully differentiated SCs, in this case p75 expression would have been upregulated. The downregulation of p75 in CMT1a SCs was already reported by Hanemann et al.,[33] chronic denervation in supernumerary SC may contribute to the downregulation. Thus, ELISAs of serum NCAM/p75 would be valuable in determining this unique SC status in CMT1a patients. Additionally, since axonal loss selectively induces p75 in myelinating SCs, the specific NCAM expression without p75 induction in CMT1a SCs may exclude significant axonal degeneration in CMT1a and thus potentially provide a differential diagnostic value from an axonal neuropathy such as AMAN-type GBS.

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## Author contributions

Study concept and design (Y. H. Kim[1], J. K. Kim, B. O. Choi, H. T. Park); data acquisition and analysis (Y. H. Kim[1], Y. H. Kim[2], S. Y. Jang, Y. K. Shin, B. A. Yoon, Y. R. Jo, D. K. Park, M. Y. Song, S. H. Nam, J. H. Kim); human sural nerve biopsy (H. Y. Shin, S. W. Kim, S. H. Kim); drafting manuscript and figures (Y. H. Kim[1], Y. H. Kim[2], J. K. Kim, H. T. Park).

## Conflict of Interest

The authors report no competing interests.

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## Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**Figure S1.** Expression profile of NCAM in the sciatic nerve after a crush injury.
**Table S1.** Partial list of the proteins identified in rat Schwann cell-derived exosomes.