Directional migration and transcriptional analysis of oligodendrocyte precursors

subjected to stimulation of electrical signal

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Running head: Guided migration of oligodendrocyte precursors

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Abstract

Loss of oligodendrocytes as the result of central nervous system disease causes demyelination that impedes axon function. Effective directional migration of endogenous or grafted oligodendrocyte precursor cells (OPCs) to a lesion is crucial in the neural remyelination process. In this study, the migration of OPCs in electric fields (EFs) was investigated. We found that OPCs migrated anodally in applied EFs, and the directedness and displacement of anodal migration increased significantly when the EF strength increased from 50 mV/mm to 200 mV/mm. However, EFs did not significantly affect the cell migration speed. The transcriptome of OPCs subjected to EF stimulation (100 mV/mm and 200 mV/mm) was analyzed using RNA sequencing (RNA-Seq), and results were verified by the reverse transcription quantitative polymerase chain reaction (RT-qPCR). A Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed that the mitogen-activated protein kinase (MAPK) pathway that signals cell migration was significantly up-regulated in cells treated with an EF of 200 mV/mm, compared with control cells. Gene ontology (GO) enrichment analysis showed the down-regulation of differentially expressed genes in chemotaxis. This study suggests that an applied EF is an effective cue to guiding OPC migration in neural regeneration, and transcriptional analysis contributes to the understanding of the mechanism of EF-guided cell migration.

Key words: electrotaxis, electric fields, migration, oligodendrocyte precursor cells, RNA-Seq
Endogenous electrical signals are involved in the development of nervous systems (28). *In vitro* studies have shown that applied small electric fields (EFs) regulate many crucial cellular behaviors, such as cell division, cell migration, and cell differentiation. Pre-clinical *in vivo* studies exhibited the potential of EFs for directing and enhancing regrowth of damaged spinal cord axons (4). Studies have also shown that the migration of primary neurons, neural stem cells, and stem cell-derived neurons can be guided by an applied electric field (7, 23, 26, 31, 42, 44). These results raise the possibility that EFs may direct axons and neural cells in central nervous system (CNS) to migrate to the lesion in order to restore electrophysiological conduction.

Glial cells have critical roles in forming myelination, maintaining homeostasis, and providing support and protection for neurons in the brain and spinal cord. The loss of oligodendrocytes (OLs) causes demyelination that impairs axon function. In response to demyelination, oligodendrocyte precursor cells (OPCs) migrate from gray and white matter to the lesion to replace mature OLs, and the differentiation of OPCs into OLs will remyelinate the axons (2, 39). OPC transplantation is an alternative approach for axonal remyelination and functional recovery in spinal cord repair (5, 13, 22). Effective directional migration of endogenous or grafted OPCs to the lesion is crucial in remyelination process. Previous studies reported that Schwann cells migrated anodally (30, 41), while neural stem cell-derived OPCs migrated cathodally in EFs (25). However, the migration of primary OPCs in EF has not been reported.

Though electrotaxis has been recognized as an effective process to direct cell migration, the mechanism that mediates the process is far from understood. A number of studies have been performed to explore molecules that may signal EF-guided cell migration (7, 23, 25, 31, 41, 42, 44, 46, 47). When mammalian epithelial cells were subjected to EF stimulation, extracellular-signal-regulated kinase (ERK1/2) was activated and polarized, and actin polymerization was induced at the leading edge of the migration cell (47). This study suggested...
that EF-polarized membrane lipid domains and epidermal growth factor (EGF) receptors cause asymmetric signaling that activates mitogen-activated protein kinase (MAPK) and induced directional cell migration. In recent work, we showed the significantly changed genes of an MAPK pathway in EF-treated Schwann cells compared with non-treated control cells (41). Additionally, this study explored differentially expressed genes (DEGs) in focal adhesion and actin cytoskeleton pathways that regulate cell migration in EFs. Next-generation RNA sequencing (RNA-Seq) is a high-throughput methodology and provides an efficient approach to identifying signaling pathways systemically. So far there has been no systematic RNA-Seq analysis on the determination of how electrostimulation of OPCs leads to their anodal migration mechanistically.

In this study, we applied the EF on the cultured OPCs and investigated the impact of EFs on cell migration direction and speed. We also studied the profile of DEGs in EF-treated OPCs in comparison with non-treated control cells using RNA-Seq. We hypothesize that the migration of OPCs can be guided by EF and some DEGs are involved in the regulation of EF-directed cell migration. The study of the migratory behavior of OPCs in EFs may lead to the application of EFs for guided migration of OPCs to damaged neural tissue in order to remyelinate regenerated axons. The dataset of gene expression provides more insight into the investigation of the regulatory mechanism of EF-guided cell migration.

MATERIALS AND METHODS

Culture of OPCs. All procedures involving animals in this study were approved by the Institutional Animal Care and Use Committee (IACUC) and completed at Wichita State University in Wichita, Kansas. The culturing of OPCs was performed as reported previously (24, 43). In brief, the cerebral cortexes were isolated from the brains of postnatal rats (day 1–2) after they were sacrificed. The cortex tissues were triturated gently through a syringe with needle. The tissue suspension was passed through a 70 mm nylon cell strainer (BD Falcon, Durham,
The flow-through was collected and cultured for about seven days. The OPCs were then isolated from the mixed-cell culture layer by mechanically shaking the cell culture flasks for about 24 h in an incubator at 37°C. The collected OPCs were cultured in an OPC growth medium (DMEM, Lifetechnologies, Grand Island, NY) with Sato media (DMEM, transferrin, BSA, progesterone, putrescine, sodium selenite [Sigma-Aldrich, St. Louis, MO]), 1% penicillin-streptomycin, and 2 mM L-glutamine (Life Technologies, Grand Island, NY). Insulin, D-biotin, sodium pyruvate, N-acetyl cysteine (Sigma-Aldrich, St. Louis, MO), trace elements B (Mediatech Inc., Manassas, VA), PDGF, and bFGF (Peprotech, Rocky Hill, NJ) were also supplemented in the medium.

Migration of OPCs in EF and Time-Lapse Imaging. To investigate the migration of OPCs in EFs, the OPCs were grown in a glass chamber that was constructed using coverslips with dimensions of 30 mm × 0.8 mm × 0.15 mm (44, 46). The chamber was coated with poly-L-lysine (100 ug/ml, Sigma-Aldrich, St. Louis, MO) and laminin (20 mg/ml, Sigma-Aldrich, St. Louis, MO). To apply EFs to the cultured cells in the chamber, agar-salt bridges (filled with Steinberg’s solution gelled with 1% agar) were used to connect silver-silver chloride electrodes in beakers of Steinberg’s solution and pools of cell culture medium. After the cells were cultured in the chamber for 24 to 36 hours, the OPCs were subjected to EF stimulation (50 mV/mm, 100 mV/mm, and 200 mV/mm). The migration of OPCs was recorded with a time-lapse microscope (Zeiss Axio Observer microscope) placed in a plastic incubator with 5% CO₂ at 37°C. Sterile conditions were maintained throughout. The time-lapse image recording was used to record cell migration using ZEN 2011 imaging microscope software, and images were taken by digital camera (AxioCam MRm Rev.3 with FireWire). The migration of cells was recorded for 1.5 hours, and then the EF power was switched on and the cell migration was recorded for an additional 1.5 hours. After this, the EF polarity was reversed without changing the field strength, and cell migration was recorded for 1.5 more hours.
Analysis of Cell Migration. The time-lapse images were analyzed using NIH ImageJ software (National Institutes of Health, Bethesda, MD). The angle at which each cell moved with respect to the EF direction was quantified (44). The mean directedness of total cell movement was calculated from the equation \[ \sum \frac{\cos \theta}{n} \], where \( \theta \) is the angle between the field vector and the cellular translocation direction, and \( n \) is the total number of cells. The net displacement was the distance of cell migration along the field line. The cell migration speed was calculated from the full distance of cell migration in a given time.

RNA Sequencing. For the RNA-Seq study, 100,000 cells were seeded in each cell culture chamber. Three independent experiments were carried out for each experimental condition (0mV/mm, 100mV/mm and 200mV/mm). Total RNA of the cells in the chamber without EF stimulation or with EF (100 mV/mm and 200 mV/mm) stimulation for 1.5 hours was extracted using an RNeasy Mini Kit (Qiagen) according to the supplier's protocol.

Total RNA samples were examined with Qubit and Agilent TapeStation for quality and quantity. Sequencing libraries were constructed using 500 ng of the total RNA. The sequencing library construction process included mRNA purification and fragmentation, cDNA synthesis, end repair, 3'-end adenylation, adapter ligation, and polymerase chain reaction (PCR) amplification. The constructed sequencing libraries were then validated and quantified with real-time PCR, Qubit, and TapeStation assays. The libraries were indexed with barcode sequences and sequenced in a multiplexed fashion. An Illumina HiSeq 2500 NGS system at the University of Kansas Genome Sequencing Core was used to generate single-end, 100-base sequence reads from the libraries. Base calling was carried out by Real-Time Analysis (RTA) software. The base call files (bcl files) were demultiplexed and converted to compressed FASTQ files by CASAVA. Samples were analyzed in biological triplicates.

The sequenced reads from each sample were aligned against the reference rat genome (Rnor 5.0.75) using Tophat2 (version 2.0.12). The differential gene expression between groups
(control vs. 100 mV/mm, control vs. 200 mv/mm, and 100 mv/mm vs. 200 mV/mm) was analyzed using Cuffdiff (3) (version 2.1.1) with default parameters. Multiple hypotheses testing correction was performed using the Benjamini Hochberg false discovery rate (FDR) method (9). Genes with q values ≤ 0.05 were considered significant.

Gene Ontology and signaling pathway enrichment analysis. Gene ontology (GO) functional enrichment analysis and signaling pathway analysis of the significantly changed genes were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (10). Significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (15, 16) were identified at the cutoff criteria of P < 0.05 after the Benjamini-Hochberg multi-test correction.

Real-Time PCR. The total RNA of the OPCs was extracted using an RNeasy Micro Kit (Qiagen, Germantown, MD) according to the supplier’s protocol. The complementary DNA (cDNA) was reverse-transcribed from the total RNA using a High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Grand Island, NY) according to the manufacturer’s protocol. The reverse transcription quantitative polymerase chain reaction (RT-qPCR) was performed using the Power SYBR® Master Mix by the StepOnePlus™ RT-qPCR System (Life Technologies, Grand Island, NY) at 95°C for 10 minutes, and then 40 cycles at 95°C for 15 seconds, followed by 60°C for 60 seconds. Gene transcription was normalized in relation to transcription of the housekeeping rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The 2^ΔΔCt method was used to calculate the relative gene expression for each target gene. Primers used in the RT-qPCR are provided in Table 1.

Statistics. Statistical analysis was conducted to assess the cell migration data using a two-tailed Student’s t-test. A p value of 0.05 was considered to be statistically significant. Data are expressed as means ± standard deviation. At least three independent experiments were performed for each condition.
RESULTS

OPCs Migrate Anodally in Applied EFs. OPCs showed typical elliptical morphology with short processes, as we reported previously (24, 43). Most OPCs showed bipolar morphology at early culture stage. We reported that the OPCs with this typical morphology were labeled with anti-A2B5 antibody and anti-PDGFRα antibody. The differentiation of OPCs into oligodendrocytes was confirmed by anti-O4 antibody and anti-MBP antibody (24, 43). In this study, the cells with the typical morphology of OPC were recognized and migration of OPCs with or without EF stimulation were tracked. The OPCs migrated randomly without EF stimulation (Figures 1A–1D), while they migrated toward the anode in an EF of 200 mV/mm (Figures 1G and 1L). When EF polarity was reversed, the cells migrated in the opposite direction (to the new anode pole) (Figures 1H and 1L). In Figures 2A–2C and 2D–2F, each frame shows the superimposed migration tracks of 47 and 61 OPCs from control and EF groups (200 mV/mm), respectively. The position of all cells at t = 0 minute is represented by the origin (0, 0). Each line represents the migration track of one single cell over a 1.5-hour period. Cells subjected to EF stimulation (200 mV/mm) showed clear anodal migration. A circular histogram (rose diagram) showed the distribution of migration cells. The biased distribution of cells in the EF (200 mV/mm) indicates their anodal migration (Figure 2E').

To confirm the migration of OPCs to the anodal pole in EFs, cell migration was recorded before and after reversal of the EF polarity. OPCs migrated toward the anode pole in an EF of 200 mV/mm (Figure 1G, 2E). After 1.5 hours, the reversal of EF polarity reversed the cell migration in the direction of the new anodal pole (Figures 1H and 2F). Quantification of migration directedness and displacement along the field line confirmed the clear reversal of cell migration in EFs of 100 mV/mm and 200 mV/mm.

The cell migration velocities of cells before and after exposure to EFs were measured and compared (Figure 3C). Though the mean migration speeds of the cells decreased at different
field strengths in comparison with that before EF stimulation, the changes were not significant. Additionally, reversal of the EF poles did not significantly alter the cell migration speed.

**OPCs Migrate Anodally in Voltage-Dependent Manner.** When the strength of EF stimulation to the cells was raised from 50 mV/mm to 200mV/mm, the directedness of the anodal cell migration increased from $-0.07 \pm 0.05$ to $-0.50 \pm 0.14$ (Figure 3A). The quantification of net displacement of the cells along the field line also showed an anodal migration pattern. For cells that were stimulated for 1.5 hours, the net displacement of cells in EFs of 50 mV/mm, 100 mV/mm, and 200 mV/mm was $-4.41 \pm 7.55\mu m$, $-13.48 \pm 5.44\mu m$, and $-21.27 \pm 5.41 \mu m$, respectively (Figure 3B). These results demonstrate that the directedness and displacement of anodal migration increased when the EF strength or stimulation duration increased.

**Analysis of Differentially Expressed Genes and GO Categories for the Control and EF-Stimulated OPCs.** Based on the threshold of significantly differential expression as fold change $>2$ and $q < 0.05$, we identified 16 up-regulated and 13 down-regulated genes in cells treated with an EF of 100 mV/mm versus control cells, 53 up-regulated and 10 down-regulated genes in cells treated with an EF of 200 mV/mm versus control cells, and 26 up-regulated genes in cells treated with an EF of 200 mV/mm versus cells treated with an EF of 100 mV/mm.

We studied the differential gene expression between cells with and without EF stimulation using the RNA-Seq approach with next-generation sequencing platforms and verification by RT-qPCR. To explore the functions of DEGs in the comparisons of control and EF-stimulated cells, we performed the GO term enrichment analysis. In the comparison of groups with an EF of 100 mV/mm stimulation and without EF stimulation, the significantly enriched GO terms of down-regulated DEGs in the categories of biological process, cellular component, and molecular function were identified (Figure 3). In the category of biological process, DEGs were significantly enriched in inflammatory response, immune response, response to wounding, defense response, behavior, taxis, chemotaxis, neutrophil chemotaxis, leukocyte chemotaxis, and cell
chemotaxis. In the category of cellular component, the DEGs were significantly enriched in the extracellular region, extracellular space and extracellular region part. In the category of molecular function, DEGs were significantly enriched in cytokine activity, chemokine activity, and chemokine receptor binding.

A comparison of groups with an EF of 200 mV/mm and those with non-EF stimulation showed that the significantly enriched GO terms of both down-regulated and up-regulated DEGs were identified (Figure 4). A significantly enriched biological process in the down-regulated DEGs is the immune response. Significantly enriched cellular components in down-regulated DEGs are the extracellular region, extracellular space, and extracellular region part. Significantly enriched molecular function in down-regulated DEGs is cytokine activity. Significantly enriched molecular functions in up-regulated DEGs are transcription regulator activity, DNA binding, transcription factor activity, sequence-specific DNA binding, double-stranded DNA binding, and structure-specific DNA binding. In the category of biological process, there are 27 significantly enriched GO terms of up-regulated DEGs, for example, regulation of transcription, negative regulation of molecular function, negative regulation of catalytic activity, response to organic substance, and response to protein stimulus. A comparison of groups with EFs between 200 mV/mm and 100 mV/mm identified the significantly enriched GO term of up-regulated DEGs. The significantly enriched biological process in up-regulated DEGs is response to protein stimulus.

**Differentially Regulated KEGG Pathways between Control Groups and EF-Treated OPCs.**

KEGG pathway analysis showed the participation of differentially expressed genes in multiple cellular signaling pathways. In the comparison of groups with EFs of 100 mV/mm and those with non-EF stimulation, four pathways—cytokine-cytokine receptor interaction, hematopoietic cell lineage, chemokine signaling, and NOD-like receptor signaling pathways—were down-regulated (Figure 5). In the comparison of groups with EFs of 200 mV/mm and those with non-EF stimulation, cytokine-cytokine receptor interaction and hematopoietic cell lineage pathways were
down-regulated for cells subjected to EF stimulation of 100 mV/mm, while the MAPK signaling pathway was up-regulated for cells subjected to EF stimulation of 200 mV/mm (Figure 6). The up-regulated genes in the MAPK signaling pathway are *Fos*, *Dusp2*, *Gadd45a*, *Gadd45b*, *Gadd45g*, and *Nr4a1*.

**RT-qPCR validation of RNA-Seq data.** To confirm the results of differentially expressed gene profiling, RT-qPCR validation was performed for ten significantly changed genes composed of seven up-regulated and two down-regulated genes (Table 2). The expression of all genes tested in the RT-qPCR validation had the same trends as those tested in the RNA-seq. Spearman’s correlation of log$_2$ transformed data from the RNA-Seq and RT-qPCR showed a significant positive correlation between these two datasets ($R = 0.968$, $P < 0.001$), thereby confirming the RNA-Seq data.

**DISCUSSION**

The potential role of EFs in neural regeneration has been proposed in recent studies (7, 26, 31, 44). EFs may function as a guidance cue to direct migration of endogenous or transplanted primary neural cells to the lesion in order to re-establish functional connection. Because the directional migration of neural cells is critical in the process of neurogenesis, a number of studies have been performed to investigate the effect of electrical cue on the migration of primary neurons and neural stem cells. Hippocampal neurons with bipolar morphology at an early culture stage are very motile, and their migration can be guided toward the cathode pole in EFs. Embryonic stem cell-derived motor neurons also showed bipolar morphology with short processes, and they migrated to the cathode in EFs (26). Neural stem cells and neural stem cell-derived OPCs (NSC-OPCs) migrated to the cathode in EFs (23, 25). In previous studies, the migration of neural stem cells and NSC-OPCs from explants of rat lateral ganglionic eminence and oligospheres, respectively, in EFs was investigated. The cells moved out of the explants or oligospheres and migrated toward the cathode. In contrast to
neurons and neural stem cells, Schwann cells migrated to the anode when they were subjected
to EF stimulation (30, 41). In this study, we observed the EF-directed anodal migration of OPCs. These studies showed that glial cells responded to EF stimulation and migrated in the opposite
direction to neurons in EF.

In this study, we found that the directedness and net displacement of anodal migration of
OPCs were enhanced when the field strength was increased. The voltage-dependent migration
behavior was also observed in the studies of neurons and neural stem cells (23, 44). Thresholds
at which cells responded to EF stimulation for directional migration varied among cell types. The
EF strength that initiated directional migration of avian neural crest cells was as low as 7
mV/mm (11, 33). The threshold for an EF to induce migration for hippocampal neurons and
Schwann cells was about 100 mV/mm. In this study, we found clear anodal migration of OPCs
in EFs of 200 mV/mm but insignificantly directed migration in EFs of 100 mV/mm. This indicates
that the threshold effectiveness of OPCs should lie between these two values. In response to
EF stimulation, both Schwann cells and OPCs migrated to the anode pole. OPCs are more
sensitive to EF stimulation than Schwann cells. The directedness of Schwann cell migration
after one hour and two hours in an EF of 200 mV/mm was 0.25 and 0.41, respectively, both of
which were lower than the directedness (0.5) of OPCs after 1.5 hours in the EF.

In a previous study, we showed the reduction of the migration speed of Schwann cells
subjected to a low EF voltage (50 mV/mm). However, the migration speed of Schwann cells did
not change significantly in EFs with higher voltages (100 mV/mm and 200 mV/mm) compared to
those without EF stimulation. This observation suggests that low voltage may retard the cell
migration speed because it cannot effectively change the cell migration direction. In this study,
though the migration speed of OPCs in EFs decreased slightly from those without EF
stimulation, the change of speed was not statistically significant.

In this study, we investigated the transcriptome of OPCs subjected to EF stimulation at two
field strengths (100 mV/mm and 200 mV/mm) using RNA-Seq. KEGG pathway analysis
explored the significantly changed molecular pathways. The MAPK pathway that regulates various cellular functions—including cell proliferation, differentiation, and migration—was significantly up-regulated in the EF-treated cells (200 mV/mm) compared with control cells. However, the significant up-regulation of MAPK pathway was not observed in cells treated with a lower EF (100 mV/mm). A number of studies have investigated the MAPK signaling pathway for regulating cell motility (6, 20, 26, 32, 40). Reports indicate that the migration of epithelial cells, fibroblasts, and vascular smooth muscle cells was regulated by the activity of the MAPK cascade (8, 12, 20, 29, 45). It was also reported that EFs can activate members in the MAPK pathway and therefore mediate directional cell migration. When corneal epithelial cells were exposed to physiological EFs, ERK was activated and F-actin accumulated at the leading, cathodal-facing side of the cell (47). In this study, we found the up-regulation of an MAPK pathway in cells treated with EFs of 200 mV/mm compared with non-treated control cells. The up-regulated genes in the pathway are *Fos, Dusp2, Gadd45a, Gadd45b, Gadd45g, and Nr4a1*. NR4A1 is a member of the steroid-thyroid hormone-retinoid receptor superfamily and functions as a nuclear transcription factor. It is involved in cell differentiation proliferation and apoptosis. When NR4A1 migrates to mitochondria, it can mediate the function of BCL-2 (B-cell CLL/lymphoma 2) to trigger apoptosis. A previous study has shown that NR4A1 regulated cell migration and acted as an antimigratory factor in normal mammary epithelial and breast cancer cell lines (1). In this study, NR4A1-overexpression in normal epithelial cells and breast cancer cells reduced cell migration ability. PAI-1 is a member of the serine proteinase inhibitor (serpin) superfamily and the major regulator of pericellular plasmin generation. It mediates cell migration by modulating interactions between vitronectin, and either the uPA receptor (uPAR) or integrins (17, 19, 27, 35, 38). FOS proteins are regulators of cell proliferation, differentiation, and transformation. Additionally, c-FOS/AP-1 DNA-binding activity controls PAI-1 gene expression, and therefore, FOS proteins may regulate cell motility. A previous study showed that antisense *c-fos* transcripts effectively reduced PAI-1 induction and growth factor-stimulated cell motility.
This study suggested that the expression of both PAI-1 and c-fos genes is necessary for wound-initiated normal rat kidney (NRK) epithelial cell migration (21). Increased expression of Gadd45a is associated with stressful growth arrest conditions or DNA damage. In response to environmental stresses GADD45A mediates activation of the p38/JNK pathway via MTK1/MEKK4 kinase. It was reported that GADD45A maintained cell-cell adhesion and cell-contact inhibition by regulating β-catenin subcellular distribution (14). One recent study demonstrated that GADD45A regulates adhesion and migration of mouse embryonic fibroblast (MEF) cells in vitro (34). Gadd45a null MEF cells (Gadd45a-/-MEFs) showed significantly increased adhesion and migratory ability in vitro.

In this study, we showed the up-regulation of Gadd45a in cells subjected to EF stimulation of 200 mV/mm compared with control cells. The up-regulation of Gadd45a may be the result of the response of OPCs to EF stimulation. However, we did not observe reduced cell motility in EF-stimulated cells. In a previous study, we showed the significantly changed MAPK pathway in Schwann cells after they were treated with EFs (41). Similar to what we found in this study, the up-regulation of Fos, Dusp2, Gadd45a, Gadd45b, and Nr4a1 was also observed in Schwann cells when treated with EFs. However, further studies are needed to determine the function of these genes in the regulation of EF-directed cell migration.

In this study, we analyzed the significantly enriched GO terms of DEGs for OPCs subjected to different electric field strengths. In a comparison of groups receiving EF stimulation of 100 mV/mm versus those receiving non-EF stimulation, the significantly enriched GO terms of down-regulated DEGs in the categories of the biological process were identified. DEGs were significantly enriched in taxis and chemotaxis. The chemokine genes—Ccl3, Ccl4, Cxcl1, and Cxcl2—were significantly down-regulated for cells subjected to EFs of 100 mV/mm compared with control cells. However, only Cxcl2 was down-regulated when the cells were subjected to a higher EF strength (200 mV/mm). Chemokines are classified into four subgroups—CXC, CX3C, CC, and XC—depending on the number and spacing of the first two conserved cysteine
residues. The interaction of chemokine and cell-specific expression of chemokine receptors induces directed chemotaxis. Chemokines play a role in spinal cord development by regulating the migration of oligodendrocyte precursors. A previous study showed that the interaction of chemokine CXCL1 and its receptor CXCR2 patterned development of the spinal cord. CXCL1 inhibited oligodendrocyte precursor migration by signaling its receptor CXCR2 (37). Unlike the astrocyte, report of the secretome of OLs is rare. A previous study investigated the secretome of human OLs generated from human neural stem cells by transduction with the gene encoding the Olig2 transcription factor (18). This study showed that the OLs secreted CXCL1, CXCL2, and IL-6 and expressed the corresponding receptors. In the current study, we observed the down-regulation of a number of chemokines in EF-treated OPCs compared with non-treated cells. This result may indicate that the generation of chemokines may be suppressed by EFs, and chemotaxis and electrotaxis may be regulated differently.

In this study, the RNA-Seq was used to systematically detect the DEGs by comparing the cells subjected to EF stimulation and the control cells. We studied the DEGs using GO term and KEGG pathway analysis and verified a number of genes that may be important in regulation of EF-guided cell migration. However, current experiments are not able to determine how these genes regulate EF-dependent cell migration. In the future studies, we will focus on a number of genes of interest and investigate the function of these genes in regulating cell migration in EF using transgenic animals and cell lines.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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**Figure legends**

Figure 1. OPCs migrating anodally in EFs. (A–D) Random migration of cells during 4.5-hour period without EF stimulation. See also supplementary material video 1. (E–G) OPC migration in EF of 200 mV/mm). Cells migrated randomly without EF stimulation in first 1.5 hours. Cells migrated to anode (left) with EF stimulation in following 1.5 hours. Then cells migrated to new anode pole when EF polarity was reversed. See also supplementary material video 2. Outlines of labeled cells in (B) and (F) highlight cell positions in (A) and (E), respectively. Outlines of labeled cells in (C) and (G) highlight cell positions in (B) and (F), respectively. Outlines of labeled cells in (D) and (H) highlight cell positions in (C) and (G), respectively. Scale bar: 50 µm.

Figures (I, J) and (K, L) are schematics showing relative cell translocation and migration direction demonstrated in Figures (A–C) and (E–G), respectively. Black, gray, and black dash lines indicate cell migration in first 1.5 hours, second 1.5 hours, and third 1.5 hours, respectively. Dots represent cell centers, and arrows represent direction of cell migration.

Figure 2. Directedness, net displacement, and migration speed of OPCs in EFs. (A–C) Analysis of OPC migration pathway. (A–C) Net translocation of OPC random migration during 4.5-hour migration period. (D–F) Net translocation of OPCs during 4.5-hour migration period including
1.5-hour random migration (D), the following 1.5-hour migration in EF of 200 mV/mm (E), and 1.5-hour migration in post-polarity reversal electric field of 200 mV/mm (F). Cell migration paths determined by video monitor tracings. Position of all cells at t = 0 min represented by origin position (center of frame), with migratory track of each cell at 90 min plotted as single line on graph. Each arm of axes represents 130 µm of translocation distance. (A’–C’) and (D’–F’) Circular histograms for cells in (A–C) and (D–F), respectively. OPCs show clear biased distribution toward anode in electric field of 200 mV/mm, indicating anodal migration of cells. Range of interval is 10 degrees. (G) Average cosine (directedness) increased when field strength increased from 50 mV/mm to 200 mV/mm. Reversal of EF polarity reversed cell migration direction. (H) Net displacement of cells along field line of EFs. Net cathodal displacement of cells became more significant when EF was increased from 50 mV/mm to 200 mV/mm. Reversal of EF polarity reversed cell displacement along field line of EFs. (I) Analysis of cell migration speed in EFs. EFs did not change cell migration velocity. *, p < 0.05, compared with migration before EF was switched on. #, p < 0.05, compared with migration before EF polarity reversal.

Figure 3. Significantly enriched GO terms of DEGs in EF-treated (100 mV/mm) OPCs in comparison with control cells. MF, Molecular Function; CC, Cellular Component; BP, Biological Process. The terms were significantly enriched with the differentially expressed genes, i.e. p values < 0.05 after Benjamini-Hochberg multi-test correction.

Figure 4. Significantly enriched GO terms of DEGs in EF-treated (200 mV/mm) OPCs in comparison with control cells. MF, Molecular Function; CC, Cellular Component; BP, Biological Process. Term was considered to be enriched significantly when p values of Benjamini test were < 0.05.

Figure 5. KEGG pathway analysis of differentially expressed genes in multiple cellular signaling pathways between control group vs. EF of 100 mV/mm and control group vs. EF of 200 mV/mm.

Figure 6. MAPK signaling pathway. Significantly up-regulated genes labeled with circle.
Hematopoietic cell lineage: 3/83
NOD-like receptor signaling pathway: 3/58
Chemokine signaling pathway: 4/179
Cytokine-cytokine receptor interaction: 6/219

MAPK signaling pathway: 6/260

KEGG pathway of down-regulated genes
KEGG pathway of up-regulated genes

Number of DEGs

100mV/mm vs. Control
200mV/mm vs. Control
Table 1. Primers used in RT-qPCR.

<table>
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<th>Gene</th>
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<th>Reverse (5'-3')</th>
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### Table 2. RT-qPCR validation

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