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The neurite growth inhibitory protein Nogo-A has diverse roles in adhesion and migration

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Next to its well-established role as a myelin-associated neurite growth-inhibitory protein, Nogo-A more recently has emerged as an important modulator of adhesion and migration. However, Nogo-A’s function in adhesion and migration is diverse and strongly dependent on a complex orchestration of cell-intrinsic as well as extrinsic factors. In this letter we summarize the current knowledge on Nogo-A’s role in adhesion and migratory mechanisms. Additionally, we revisit an earlier hypothesis concerning a putative role for Nogo-A as regulator of early postnatal cerebellar granule cell migration. Pulse labeling of proliferating cerebellar granule neurons in early postnatal wild-type and Nogo-A-null mice enabled us to follow their migration dynamics over multiple days. Neither migration speed, nor cell proliferation, or layer area sizes were influenced by Nogo-A deletion, hence suggesting another role for early postnatal Nogo-A expression in the premigratory zone of the external granule layer.

A key mechanism underlying cell migration of nucleated mammalian cells is the concerted modulation of adhesion and deadhesion during protrusion of the front, translocation of the cell body, and retraction of the rear.1 These polarized actomyosin-driven cell shape alterations can be modulated by cell-intrinsic as well as a wide variety of extrinsic factors, ranging from different extracellular matrix components to cell–cell junctions.2 Consequently, different cell types or the same cell type in different microenvironments can show different or even opposite migration responses following modulation of the same adhesion/migration-relevant signaling molecule. The different migration-modulating functions of the neurite outgrowth inhibitory membrane protein Nogo-A may reflect this multivalent impact of intrinsic and extrinsic factors on migratory behavior.

Nogo-A was initially identified as a myelin-associated inhibitor of neurite outgrowth, but also as an inhibitor of fibroblast spreading.3 For many years the main focus of research was almost exclusively directed toward the neurite outgrowth inhibitory and growth cone collapsing effects of Nogo-A. In 2003, Oertle and colleagues for the first time described a Nogo-A-specific functional domain termed Nogo-A-Δ20 that proved to be responsible for the previously observed spreading inhibitory effects of Nogo-A in 3T3 fibroblasts.4 Interestingly, this domain did not require the earlier identified receptor NgR1 that was shown to bind to the functional Nogo-66 domain in the C-terminal RTN-homology domain.5 Observing this strong anti-adhesive effect in a number of different cell types apart from 3T3s, including Chinese hamster ovary (CHO) cells as well as primary neurons, Oertle et al. hypothesized that Nogo-A, next to its neurite outgrowth inhibitory and growth cone collapse-inducing functions, could play additional roles in adhesion-dependent processes such as cell migration.4

It was only in the recent 6 y that this adhesion/migration-modulating function was rediscovered. Hence, the widespread involvement of Nogo-A in adhesion and migration-related events and its complexity are becoming increasingly evident: Recombinant Nogo-A-Δ20 but not Nogo-66 inhibits spreading and migration of primary brain microvascular endothelial cells in vitro. This RhoA-ROCKII-Myosin-II-pathway-dependent process can be blocked using anti-Nogo-A antibodies and proved to be important for CNS angiogenesis in vivo.6 In an entirely different system, the rostral migratory stream in the adult mouse brain, Nogo-A was shown to be expressed in immature neuroblasts while NgR1 was found in germinal astrocytes.7 Interestingly, the Nogo-66/NgR1 interaction reduces proliferation of the neural stem cells, while the Nogo-A-Δ20 domain promotes migration of the neuroblasts toward the olfactory bulb. The latter is achieved via activation of the RhoA-ROCK pathway without requiring the Nogo-66 receptor NgR1.7 Also, embryonic neuronal precursors from the cortex of Nogo-A-null mice as well as neurospheres derived from wild-type mice treated with anti-Nogo-A, anti-Lingo-1, or anti-NgR antibodies showed decreased adhesion and increased motility in vitro.8

Furthermore, BrdU pulse experiments revealed a delay in the tangential migration of E12.5 cortical neurons from Nogo-A/B/C-null mice in vivo,9 as well as radial migration defects of neuronal precursors in the cortex of Nogo-A-null vs wild-type mice.8 The transient accumulation of radially migrating precursors—typically visible at embryonic day E17.5—was invisible and the expected migration to upper cortical layers at E19 was disturbed in Nogo-A-null mice.8 Interestingly, adhesion and migration of olfactory ensheathing cells (OECs), which have been linked to neural progenitor migration in the rostral migratory stream,10 is reduced upon stimulation with recombinant Nogo-66 in vitro.11 Antibody treatment against NgR1 as well as phosphatidylinositol-specific phospholipase C treatment, which is known to cleave...
Figure 1. For figure legend, please see page 453.
NgR1, led to an attenuation of the observed effects. Additionally, anti-NgR1 treatment facilitated migration of implanted OECs in a spinal cord hemisection injury model. The Nogo-66/NgR1 pathway also seems to modulate adhesion and migration of a range of immune cells in the central nervous system. Human immature myeloid dendritic cells, expressing high levels of NgR1 and NgR2, were shown to adhere less to myelin or recombinant Nogo-66 substrate than mature dendritic cells, containing lower amounts of these receptors. The downregulation of NgR1/2 thus promotes adhesion of dendritic cells to myelin, and consequently, was hypothesized to play a role in neuroinflammatory diseases such as multiple sclerosis in which peripheral immune cells come into direct contact with myelin debris. In line with these results another study revealed that NgR1/2 double-null mice show an increased leukocyte infiltration into the CNS, further supporting a possible role for NgR1s in the regulation of central nervous system immune cell migration.

Furthermore, Nogo-66 inhibits adhesion and migration of microglia via NgR1 and RhoA activation as well as Cdc42 inhibition in vitro. Since polarization and membrane protrusion formation of microglia were inhibited by recombinant Nogo-66 in vitro, these cell morphological changes putatively account for the observed mobility effects. These findings might be of specific interest for the development of demyelinating diseases, such as multiple sclerosis, where NgR was found to be expressed on the surface of macrophages/microglia. Earlier expression data from our lab showed a strong expression of Nogo-A in the premigratory external granule cell layer of the early postnatal mouse cerebellum. They raised the question whether Nogo-A might also be involved in cerebellar granule neuron (CGN) migration during maturation of the cerebellar cortex. We addressed this question by injecting P7 Nogo-A-null C57BL/6 mice and wild-type controls subcutaneously with 20 ul/g body weight of a 5 mg/ml PBS stock of 5-ethyl-2'-deoxyuridine (EdU, Invitrogen). After 24 h (n = 6 WT/5 KO), 48 h (n = 7 WT/4 KO), 72 h (n = 7 WT/6 KO), and 96 h (n = 7 WT/5 KO) the animals were sacrificed and perfused with 4% paraformaldehyde (PFA). Brains were dissected and postfixed in 4% PFA, before being transferred to 30% sucrose in 0.1M phosphate buffer. 25 μm-thick sagittal sections were cut using a cryostat and subsequently stained for nuclei (DAPI, Invitrogen) and proliferating, EdU-positive cells (Alexa 647-azide, Invitrogen) employing click chemistry as described before. 20x mosaic images of three to six sagittal sections per cerebellum were acquired using the Panoramic 250 slide scanner (3D Histech) and two regions of interest (ROIs) were set in lobules III and IV/V for detailed analysis. Representative images of the ROI in lobule III for all time points and genotypes are shown in (E). The mean numbers of EdU-positive cells per layer normalized to the total number of proliferating cells in all layers are plotted for 24 h, 48 h, 72 h, and 96 h post-EdU administration for the EGL (B), the ML (C), and the IGL (D). Due to the high density of cells in the EGL 24 h post-EdU administration, the number of EGL cells at 24 h is not counted directly but calculated using the estimation that the total number of EdU-positive cells is comparable between the 24 h and 48 h timepoints. Distances traveled by each of the cells after 48 h, 72 h, and 96 h is shown in form of a cumulative frequency distribution plot for Nogo-A-null as well as wild-type mice in (F) (no significant difference between genotypes as calculated using the Kolmogorov-Smirnov test). The mean areas of the external granule, the molecular, as well as the internal granule layers of P10 wild-type and Nogo-A-null mice are shown in (G). The mean total numbers of EdU-positive cells per region of interest for both genotypes are plotted in (H). The scale bar in (E) corresponds to 100 μm and the dotted lines indicate the following layer boundaries: EL, external granule layer; ML, molecular granule layer; IL, internal granule layer.

Figure 1 (see previous page). Proliferation and migration of early postnatal cerebellar granule neurons in Nogo-A−/− vs. wild-type mice. (A) Experimental design: P7 Nogo-A−/− mice and wild-type controls were subcutaneously injected with EdU. After 24 h (n = 6 WT/5 KO), 48 h (n = 7 WT/4 KO), 72 h (n = 7 WT/6 KO), and 96 h (n = 7 WT/5 KO) the animals were sacrificed and perfused with 4% paraformaldehyde. The brains were postfixed and cut into 25 μm sections using a cryostat. The sections were subsequently stained for nuclei (DAPI, Invitrogen) and proliferating, EdU-positive cells (Alexa 647-azide, Invitrogen). 20x mosaic images of three to six cerebellum sections were acquired using the Panoramic 250 slide scanner (3D Histech) and two regions of interest (ROIs) were set in lobules III and IV/V for detailed analysis. Representative images of the ROI in lobule III for all time points and genotypes are shown in (E). The mean numbers of EdU-positive cells per layer normalized to the total number of proliferating cells in all layers are plotted for 24 h, 48 h, 72 h, and 96 h post-EdU administration for the EGL (B), the ML (C), and the IGL (D). Due to the high density of cells in the EGL 24 h post-EdU administration, the number of EGL cells at 24 h is not counted directly but calculated using the estimation that the total number of EdU-positive cells is comparable between the 24 h and 48 h timepoints. Distances traveled by each of the cells after 48 h, 72 h, and 96 h is shown in form of a cumulative frequency distribution plot for Nogo-A-null as well as wild-type mice in (F) (no significant difference between genotypes as calculated using the Kolmogorov-Smirnov test). The mean areas of the external granule, the molecular, as well as the internal granule layers of P10 wild-type and Nogo-A-null mice are shown in (G). The mean total numbers of EdU-positive cells per region of interest for both genotypes are plotted in (H). The scale bar in (E) corresponds to 100 μm and the dotted lines indicate the following layer boundaries: EL, external granule layer; ML, molecular granule layer; IL, internal granule layer.
migration behavior of these cells during postnatal days P7 to P11. Yet the time-specific and localized expression of Nogo-A during early postnatal cerebellar development might suggest different roles for this molecule, including e.g., neurite growth or branch angle determination of parallel fibers, which is known to start simultaneously with Nogo-A expression in the premigratory EGL at around P3 to P4. Interestingly, during development, Nogo-A has been implicated in regulation of branch angle determination as well as fasciculation of dorsal root ganglion neuronal or fibroblasts on substrates in vitro comprise distinct EGL at around P3 to P4.21 Interestingly, during development, start simultaneously with Nogo-A expression in the premigratory branch angle determination of parallel fibers, which is known to during early postnatal cerebellar development might suggest differences seen above are reflecting the importance of a diverse orchestration of intrinsic and extrinsic factors in migratory behavior: interneuron migration along corticofugal axons, radial glia-guided migration of cells, homotypic sliding/chaining migration of SVZ neuroblasts or single cell migration of primary brain microvascular endothelial cells, or fibroblasts on substrates in vitro comprise distinct microenvironments which might explain the differences seen between different systems upon modulation of Nogo-A activity. It consequently seems very likely that Nogo-A's emerging role as a versatile migration modulator will be further extended in the future.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Ethical Statement
All animal experiments were performed in accordance to the guidelines of the veterinary authorities.

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