Proteomic and Functional Analysis of Neurochondrin: A Potential Therapeutic Target for Spinal Muscular Atrophy and Other Neurodegenerative Diseases

University of St Andrews

Lucy Knight Robinson

Supervised by: Dr. Judith Sleeman, School of Biology

Introduction

Spinal muscular atrophy (SMA) is an inherited neurodegenerative disease, caused by a reduction of functional **survival motor neuron (SMN) protein**. The SMN protein is multifunctional with studies showing that SMN deficiency reduces neurite outgrowth (Singh et al., 2017).

Neurite outgrowth is the process by which neurons produce new projections in response to environmental guidance cues. Despite understanding the main structures associated with neurite outgrowth, the exact mechanism remains elusive, with involvement of individual proteins being elucidated but not conclusively linked to one another. **Neurochondrin (NCDN)** is an essential neural protein suggested to regulate neurite outgrowth (Dateki et al., 2004).

SMN and NCDN have been shown to co-localise in mobile cytoplasmic vesicles of neurites in SH-SY5Y cells. Therefore, it has been suggested that SMN depletion, as in SMA, may affect the localisation and/or function of NCDN (Thompson et al., 2018). Given that both proteins are implicated in neurite outgrowth and have been shown to interact, it is of interest to determine if there is a mechanism by which both proteins co-dependently initiate or promote the process.

Hence, with the objective of identifying a mechanistic role for NCDN and/or SMN in the process, neurochondrin and SMN SILAC-derived (stable-isotope labelling by amino acids in cell culture) proteomes were compared to identify and investigate interacting proteins known to be associated with neurite outgrowth.

Results

Neurochondrin/SMN Co-interacting Protein (NSCIP) was selected from the SILAC screen as it was shown to interact with both neurochondrin and SMN. As research is unpublished, the official protein name cannot be disclosed at this time.

NSCIP has been implicated in neuritogenesis with interacting protein G-protein β2 subunit (GNB2) where constitutive expression of NSCIP induced neuritogenesis by activating the RAS-MAPK pathway in PC12 cells (REDACTED, 2006).

We were interested to explore whether neurite outgrowth could be promoted by overexpressing NSCIP in the SH-SY5Y cell line, in cells with normal NCDN expression (SH-SY5Y), and cells with reduced NCDN expression (shNCDN). Average neurite lengths were evaluated at 24- and 48-hour intervals and 24-, 48-, and 72-hour intervals respectively (Figure 1) for cells overexpressing NSCIP, and cells with normal NSCIP expression. Measurements for each respective cell line were compared.

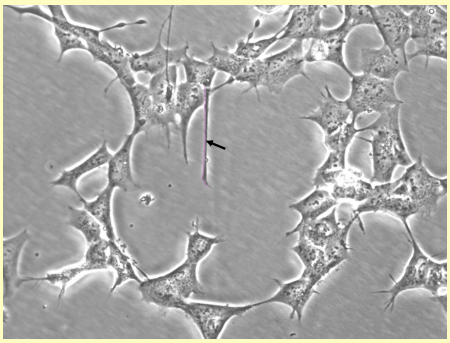

Figure 1: Representative Image Showing How Neurites were Measured. *Based on green fluorescent protein (GFP) imaging, cells transfected with NSCIP were identified. An example of a neurite overexpressing NSCIP being measured is shown by the arrow, the pink line showing where the measurement was taken from/to.*

Results - Neurite Length

Longer neurites were observed in SH-SY5Y cells overexpressing NSCIP compared to cells with normal NSCIP expression across all timepoints (Figure 2)

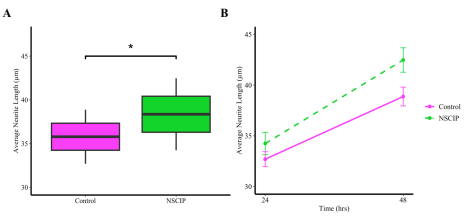

Figure 2: Increased Neurite Outgrowth in SH-SY5Y Cells through NSCIP Overexpression. *Average neurite length was recorded at 24 hours and 48 hours for cells transfected with NSCIP (NSCIP, green) and for cells not transfected with NSCIP (Control, magenta). A: A between-subjects factorial ANOVA, showed a significant main effect of NSCIP over-expression, p = 0.012, on neurite outgrowth across all timepoints. B: There was not a significant interaction between time and NSCIP overexpression.*

Longer neurites were observed in shNCDN cells overexpressing NSCIP compared to cells with normal NSCIP expression (Figure 3).

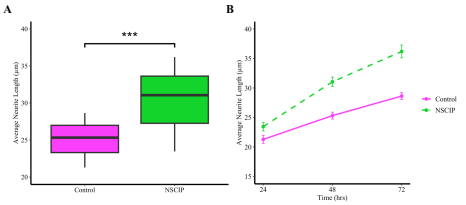

Figure 3: Increased Neurite Outgrowth in shNCDN Cells through NSCIP Overexpression. *Average neurite length was recorded at 24 hours, 48 hours, and 72 hours for cells transfected with NSCIP (NSCIP, green) and for cells not transfected with NSCIP (Control, magenta). A: A between-subjects factorial ANOVA, showed a significant main effect of NSCIP overexpression, p < 0.001, on neurite outgrowth across all timepoints. B: There was not a significant interaction between time and NSCIP overexpression.*

While transfection with NSCIP was not able to fully rescue the neurite length loss observed with neurochondrin depletion, partial compensation was observed with an increase in neurite length observed in shNCDN cells transfected with NSCIP compared to shNCDN cells not transfected with NSCIP and a control SH-SY5Y cell line (Figure 4).

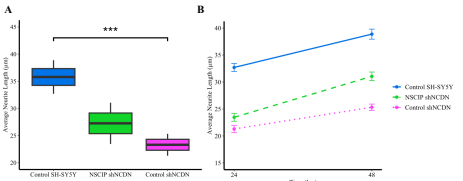

Figure 4: Overexpression of NSCIP Promotes Neurite Outgrowth and Partially Compensates for the Loss of NCDN. *Average neurite length was recorded at 24 hours and 48 hours for shNCDN cells transfected with NSCIP (NSCIP shNCDN, green) and shNCDN cells not transfected with NSCIP (Control shNCDN, magenta). This was compared to SH-SY5Y cells not transfected with NSCIP (Control SH-SY5Y, blue).*

References

Custer, S.K., Foster, J.N., Astroski, J.W. and Androphy, E.J. (2019). Abnormal Golgi morphology and decreased COPI function in cells with low levels of SMN. Brain Research, 1706, pp.135–146. doi:https://doi.org/10.1016/j.brainres.2018.11.005.

Dateki, M., Mochizuki, R., Yanai, K. and Fukamizu, A. (2004). Identification of the mouse neurochondrin promoter region and the responsible region for cell type specific gene regulation. Neuroscience Letters, 356(2), pp.107–110. doi:https://doi.org/10.1016/j.neulet.2003.11.026.

Singh, R.N., Howell, M.D., Ottesen, E.W. and Singh, N.N. (2017). Diverse role of survival motor neuron protein. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms, 1860(3), pp.299–315. doi:https://doi.org/10.1016/j.bbagrm.2016.12.008.

Thompson, L.W., Morrison, K.D., Shirran, S.L., Groen, E.J.N., Gillingwater, T.H., Botting, C.H. and Sleeman, J.E. (2018). Neurochondrin interacts with the SMN protein suggesting a novel mechanism for spinal muscular atrophy pathology. Journal of Cell Science, 131(8), p.jcs211482. doi:https://doi.org/10.1242/jcs.211482.

REDACTED (2015), REDACTED (2006), REDACTED (2011), REDACTED (2017).

Discussion

Given that NSCIP promotes neurite outgrowth with a knockdown of neurochondrin, we suggest either:

1) NCDN and NSCIP promote neurite outgrowth via distinct pathways, and therefore function of NSCIP is not regulated by neurochondrin
2) NCDN and NSCIP promote neurite outgrowth in a shared pathway, where increased NSCIP expression can partially compensate for loss of neurochondrin.

Prior experiments within the lab conducted by Thompson, 2017 showed cytoplasmic localisation of NSCIP, concentrated in the perinuclear region in SH-SY5Y cells. Additionally, while not quantifiable, the NSCIP interacting protein GNB2 was detected within the SILAC screen. **It is possible that there may be a role for NCDN in the pathway proposed by REDACTED, 2006, whereby the Ras-MAP kinase-Elk-1 cascade is activated to promote neurite outgrowth**.

An interaction between NCDN and both SMN and NSCIP has been demonstrated in this study. SMN and NSCIP have independently been implicated in regulating Golgi dynamics, which if disrupted could impact neurite outgrowth. From type I and II SMA patient-derived fibroblasts, Golgi apparatus morphology demonstrated a diffuse distribution, in SMN depleted conditions (Custer et al., 2019). A similar diffuse Golgi structure was observed in HeLa cells transfected with siNSCIP (REDACTED, 2015). Given that it has been proposed that NSCIP may be at the interface of the Golgi membrane and cytoskeleton (REDACTED, 2017), **it would be of interest to determine whether there is a mechanistic link between NSCIP and SMN in vesicular transport. Since neurochondrin has been suggested to be involved in the transport of trafficking vesicles (Thompson et al., 2018), there may be a mechanism by which the three proteins interact.**

Conclusion

The potential significance of NSCIP as a therapeutic target for SMA and other neurodegenerative diseases holds promise. Additional research is required to further examine the dynamic interplay between NSCIP, NCDN, and SMN to construct a clearer picture.

Methods

Stable SH-SY5Y cell lines with constitutive NCDN under-expression, shNCDN, and normal NCDN expression, SH-SY5Y were used. From ongoing research, a quantitative proteomic data set was obtained using SILAC comparing the interactomes of stable cell lines constitutively over-expressing NCDN and SMN.

Transfections of SH-SH5Y and shNCDN cells with NSCIP plasmid were performed, and neurite lengths evaluated using the EVOS M5000 cell imaging system at a magnification of 40X. Images were imported into Fiji (ImageJ) and neurites measured with the NeuronJ plugin.

Statistical analysis was conducted with R (version 4.2.3). In all statistical tests, the Levene and Shapiro-Wilk assumptions were violated and so data was logarithmically transformed to obtain a corrected *P*-value. Error bars are ± standard error of mean (SEM). Graphs were produced using R, ggplot2 software.

I would like to express my sincere gratitude to my supervisor, Dr. Judith Sleeman, for her invaluable guidance and support throughout my project. Additionally, I would like to extend my thanks to Lord Laidlaw and the Laidlaw Foundation for their generous funding.

LAIDLAW SCHOLARS