The role of Sip1 in development of the central and peripheral nervous systems

Sasha Machulsky[1] and Crystal D. Rogers[2]

[1]California State University Northridge, CA 91330, [2]University of California, Davis, CA, 95616

Introduction

Smad-interacting protein-1, also known as Sip1/Zeb2/ZFHX1B, is a two-handed zinc finger, homeodomain transcription factor. It is located in the cell nucleus and functions as a DNA-binding transcriptional repressor and interacts with activated SMAD proteins that transduce the signals from bone morphogenetic (BMPs) and transforming growth factor beta (TGF-**β**) proteins. The interaction between Sip1 and SMAD proteins is fundamental for transmitting BMP and TGF-**β** signals from the cell surface to the nuclei and for regulating transcriptional responses [1, 2]. Sip1 is expressed in the developing neural tissue and neural crest (NC) cells in vertebrate embryos [3]. Mutation of the Sip1 gene is known to cause embryonic defects such as craniofacial abnormalities, enteric aganglionosis (Hirshsprung's Disease), and Mowat Wilson Syndrome [2, 4].

The role of Sip1 has been previously studied in early embryogenesis during NC EMT as well as in many different types of cancer cells, however little is known about its role in neurogenesis [5]. NC cells are an ectoderm-derived embryonic cell population that gives rise to multiple derivatives. Relevant to this study, the NC cells begin in the central nervous system (CNS) and form the peripheral nervous system (PNS), which includes cell variants such as the trigeminal ganglia, dorsal root ganglia, and sympathetic ganglia [1, 4].

Here, we investigate the role of Sip1 protein during embryonic development by performing loss of function experiments to determine if Sip1 is required for the formation and differentiation of CNS and PNS derived neurons. A Sip1 translation-blocking morpholino oligomer was injected into embryos at HH8 to determine if Sip1 is necessary for development of ectodermal derivatives. Immunohistochemistry (IHC) was performed for cell-cell adhesion proteins (Ncad and Ecad), neuronal markers (Tubulin-**β**-3), and NC cells (Sox9). We determined that knocking down Sip1 at stage 8 has no effect on development of neural or neural crest cells in the midbrain, but in the hindbrain, loss of Sip1 increased Ncad and caused aberrant NC and neuronal development. Future experiments will determine if these phenotypes persist into later stages by assessing NC derivatives and specific CNS patterning and cell types. Additionally, gain of function experiments will be performed to determine if excess Sip1 has an effect on the differentiation of neurons and glia.

Methods

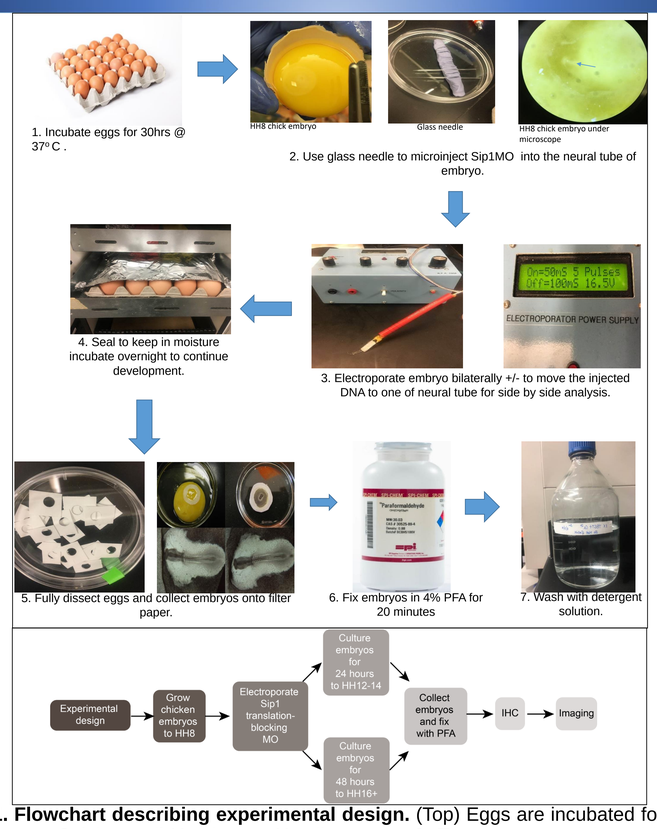

Figure 1. Flowchart describing experimental design. (Top) Eggs are incubated for 30 hours at 37 degrees Celsius until Hamburger Hamilton stage 8. Treatments are injected in ovo and electrodes are placed on either side of the embryo, negative on one side and positive on the other. DNA and morpholinos are repelled by the negative electrode and pushed into one side of the neural tube. Embryos are then wrapped tightly to prevent desiccation, and are incubated overnight (12-24 hours) until collection. After embryos are collected, whole mount immunohistochemistry is performed, embryos are imaged, embryos are cryosectioned in the transverse plane, and then sections are imaged.

Results

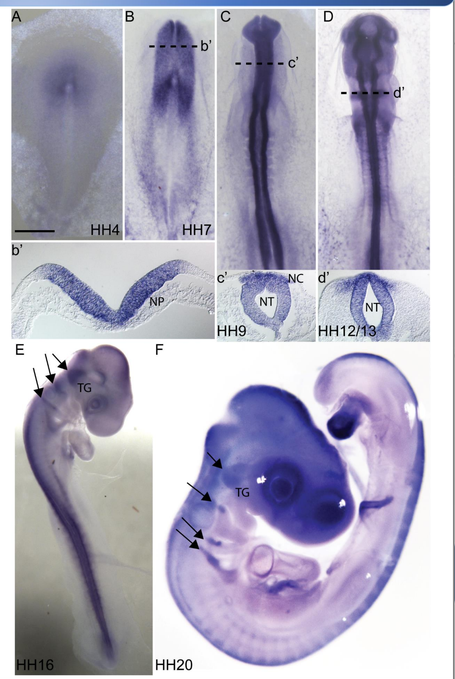

Figure 2. Figure 1. *Sip1* is expressed in the developing neural tube and neural crest. (A-F) Whole mount in situ hybridization of chick embryos at various stages using an antisense probe to *Sip1* reveals expression in the developing neural plate (NP, A, B), neural tube (NT, C-F), premigratory and migratory NC cells (NC, C, D) at stages HH4- HH20. Sections of indicated embryos at axial level marked by dashed lines (b',cf' and d'). *Sip1* is expressed throughout the neural plate, neural tube, and in migratory NC cells. (E, F) Later stage embryos (HH16, HH20) express *Sip1* in the trigeminal ganglia (TG) and other cranial ganglia (arrows). The expression of *Sip1* transcripts suggests that it may be involved in CNS and PNS development. Scale bars are 200 um (A-H) and 100 um (c'-g''). Adapted from Rogers et al., 2013.

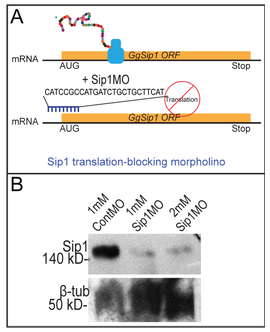

Figure 3. Verification of Sip1MO. (A) Diagram displaying mechanism of morpholino blocking the translation of Sip1 protein. (B) Western blot comparing protein from control embryos (ContMO) and those injected with two concentrations of Sip1MO demonstrating the efficient knockdown of Sip1 protein after morpholino injection. Adapted from Rogers et al., 2013.

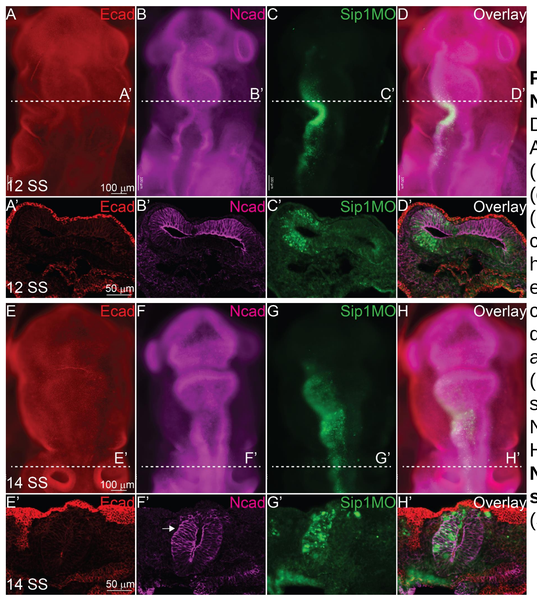

Figure 4. Loss of Sip1 increases Ncad in posterior neural tube. (A-D) IHC for Ecad expression (red; A, A', E, E'), Ncad expression (magenta, B, B', F, F'), Sip1MO (green, C, C', G, G') and overlay (D, D', H, H') in chick embryos cultured for 12 hours (A-D) and 24 hours (E-H) following injection and electroporation of Sip1MO. In contrast to previous studies demonstrating an increase in Ecad after Sip1 knockdown at stage 4 (Rogers et al., 2013), injection at stage 8 has no effect on Ecad or Ncad expression in the midbrain. However, **loss of Sip1 increases Ncad in the hindbrain at 14 somite stage** after stage 8 injection (SS) (F', arrow).

Results

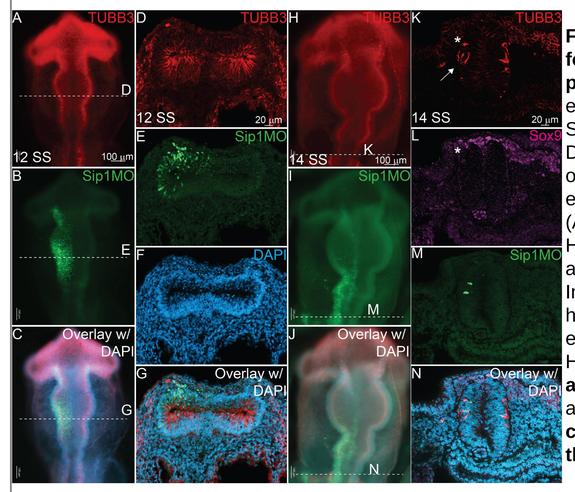

Figure 5. Sip1 is necessary for TUBB3+ neurite patterning. IHC for TUBB3 expression (red; A, D, H, K), Sip1MO (green, B, E, I, M), DAPI (F), SOX9 (L), and overlay (C, G, J, N) in chick embryos cultured for 16 hours (A-D, 12 SS) and 24 hours (E-H, 14 SS) following injection and electroporation of Sip1MO. Injection of Sip1MO at stage 8 has no effect on TUBB3 expression in the midbrain. However, **loss of Sip1 causes aberrant neurite guidance** (K, arrow) **and reduces neural crest cells** (K, M, asterisk) **in the hindbrain at 14 SS**.

Conclusion

- Sip1 is expressed at the right time and place to be involved in CNS and PNS neurogenesis.
- Loss of Sip1 at HH8 has no effect on cadherin or TUBB3 protein expression in the midbrain.
- Loss of Sip1 at HH8 increases Ncad and reduces hindbrain neural crest cells marked by SOX9 and TUBB3 and causes a disruption in neurite guidance.

- *Does increase in Ncad directly affect NC cell reduction and neuron guidance defects?*

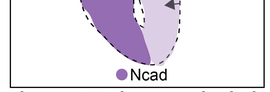

Figure 6. Diagram depicting Sip1-knockdown phenotype.

Future Directions

- Determine if Sip1 is directly regulating Ncad expression in the hindbrain.
- Identify if NC and neuronal phenotypes persist later in development.
  - *Are there craniofacial or PNS defects?*
  - *Do all CNS cell subtypes form?*
- Identify the stages at which Sip1 is required for NC, CNS, and PNS development.
  - *Sip1 is expressed in the developing CNS, NC, and differentiating ganglia*

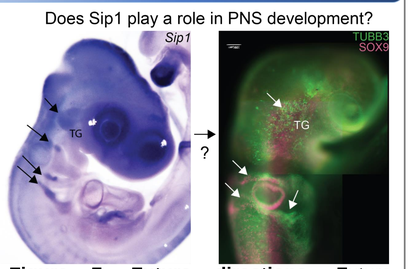

Figure 7. Future directions. Future experiments will investigate the necessity for Sip1 in CNS and PNS neurogenesis and axon guidance.

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