Deciphering glial evolution: genetic and functional characterization of ancestral glia

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Introduction

Background

Central nervous systems of bilaterian animals generally consist of two cell types – neurons and glial cells. Glia participate in almost every process taking place in the nervous system of bilaterian animals. Therefore, tracing back the first glia and elucidating its ancestral function is important for understanding the evolution of the nervous system. Histological examinations have not so far revealed any morphological sign of glial cells in *Cnidaria*, the closest outgroup to *Bilateria*.

It is thus believed that glial cells appeared after the common bilaterian ancestor had branched off from *Cnidaria* (Fig.1). However, this view has not been well examined at the genetic level.
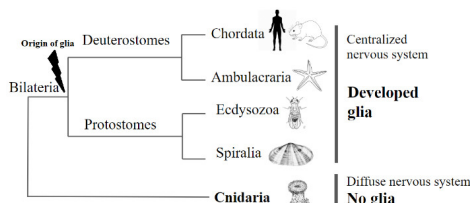

Figure 1. Current view of glial evolution

Hypothesis

Given that several bilaterian gliogenic transcription factors (TF) as well as functional genes are conserved in *Nematostella vectensis* (*Cnidaria*), I hypothesize the existence of glia-like cells in these basal metazoans.

Aims and methods

Goal: Understand evolutionary processes of glial cells at the genetic level
Aims: 1. Identify evolutionary conserved glial gene sets in *Nematostella* 2. Investigate functions of evolutionarily conserved gliogenic TFs in *Nematostella* 3. Characterize glial-marker-expressing cells in *Nematostella*

Materials and methods

- Comprehensive phylogenetic analysis of "glial" genes using a large set of genomic data of all key taxa to identify evolutionarily conserved gene sets and search for their homologs in the genome of *Nematostella*.
- Expression analysis of identified conserved key glial TFs in *Nematostella* using whole-mount in situ hybridization (WISH).
- Functional analysis of conserved glial TFs: knockdown using siRNA followed by transcriptome analysis to identify target genes and pathways; characterization of cell clusters expressing glial TFs based on recently published single-cell transcriptome data of *Nematostella* (Sebé-Pedrós et al., *Cell*, 2018).
- Functional analysis of glial TF target genes: knockdown/inhibition.
- Morphological analysis of glial-marker-expressing cells in *Nematostella* using antibodies against glial TFs and co-expressed functional and structural genes. Study design is summarized in figure 2.
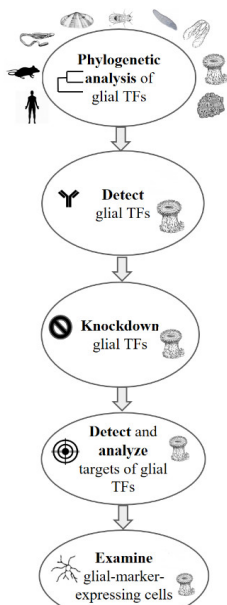

Figure 2. Experimental design

Results

GCM is the "core" gliogenic TF in *Nematostella*

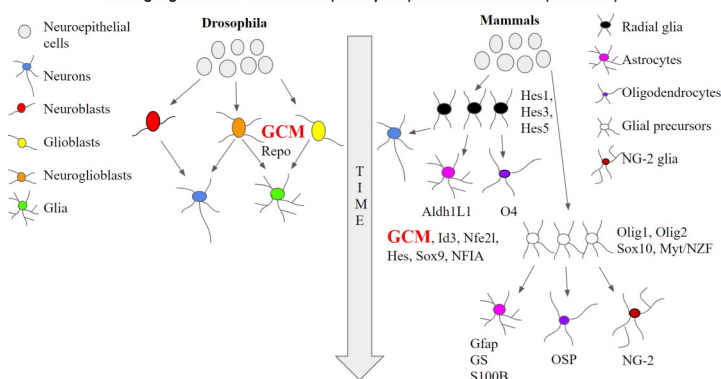**Neuro/gliogenesis in *Protostomes* (*Drosophila*) vs. *Deuterostomes* (mammals)**

Figure 3. TFs driving gliogenesis and glial-specific markers in Protostomes and Deuterostomes. *GCM* appears to be the only conserved glial TF.

Co-localization of *GCM*-expressing cells and cnidarian nervous system
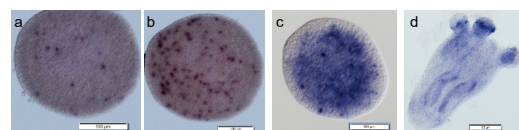

Figure 4. Expression of *GCM* at blastula (a), gastrula (b), planula (c), and early juvenile (d) stages. Scale bar - 100 μm.

GCM-expressing cells combine characteristics of both glia and neurons

GCM-containing cell clusters are characterized by the presence of **glutamate, GABA and neuropeptide metabolism** related genes, neurotransmitter receptors, and **neuronal genes.**
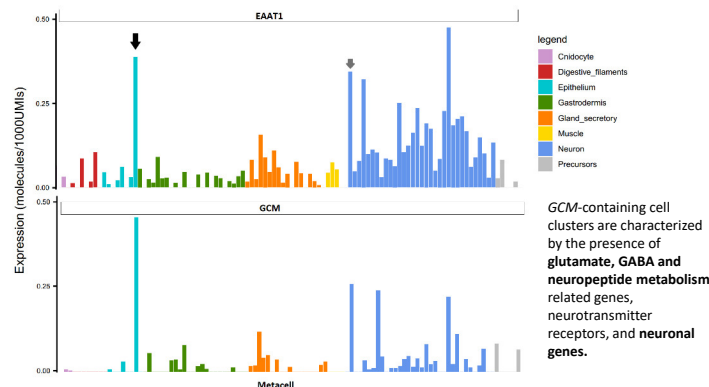
Figure 5. *EAAT1* (top) and *GCM* (bottom) expression in different cell clusters in adult *Nematostella*. Both are enriched in a particular neuronal (grey arrow) and an epithelial (black arrow) cell clusters.
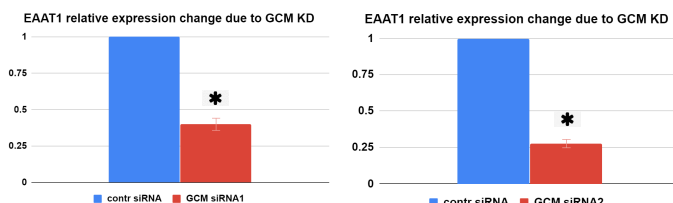

Figure 6. EAAT1 expression level change due to GCM MO knockdown using siRNA1 (left) and siRNA2 (right). Data are given mean ±SEM, n = 5,*p < 0.01, unpaired t-test. Gapdh was used as a normalizer.

Conclusions and future work

- *GCM* is the most conserved glial TF that may have a pivotal and evolutionary conserved function to instruct cells to become glial-like.
- It is not clear if the function of *GCM* in *Nematostella* is to guide neuronal differentiation or control the expression of glial functional genes, or both.
- Genome-wide transcriptomic analysis of *GCM* knockdown animals and functional analysis of *GCM* target genes will clarify the conserved function of *GCM*.
- Morphological assessment of *GCM*-expressing cells will help identify if these cells are indeed glial-like.

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