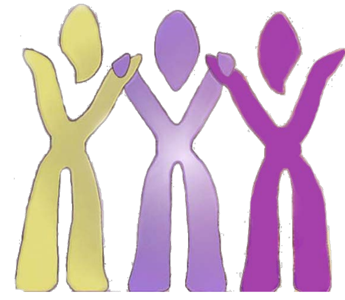
Identifying Astrocyte-Specific Targeting Ligands Through Peptide Phage Display



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BIOE

Abstract

Because lineage-specific markers for astrocytes have yet to be discovered, there is little understanding of their development, location, and cell-to-cell interaction. By performing *in vitro* phage display with primary astrocytes as a target, the Pun Lab hopes to identify peptides that bind specifically to astrocytes isolated at distinct phases of brain development. This is in the hopes that we will be able to eventually synthesize targeted imaging probes to visualize these populations *in vivo*.

Background

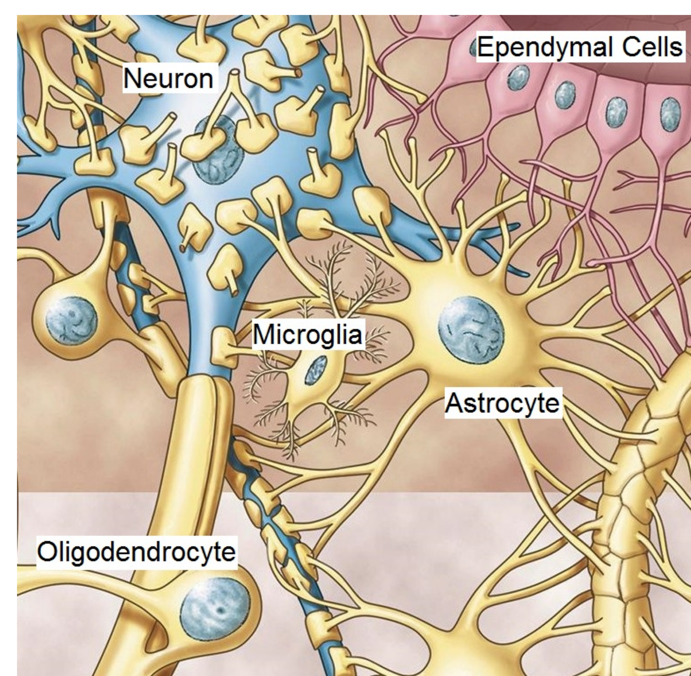


Figure 1. The cellular environment of the brain. *Image credit: Pearson Education.*

Astrocytes:

- Provide nutrients to neurons
- Create blood-brain barrier
- Maintain homeostasis following injury
- Neuronal maturation
- Synapse formation
- Neurogenesis regulation

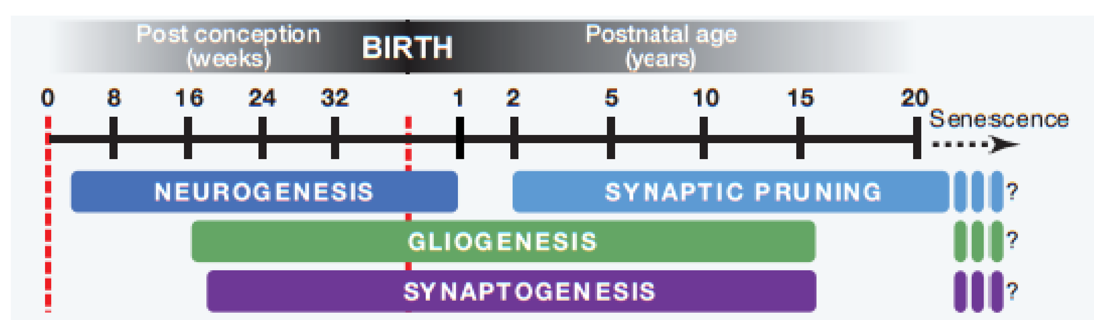


Figure 2. Timeline depicting the differential development of brain cells of various lineages. Adult neuro- and gliogenesis is not well understood. *Image credit: Liddelow and Barres, 2015.*

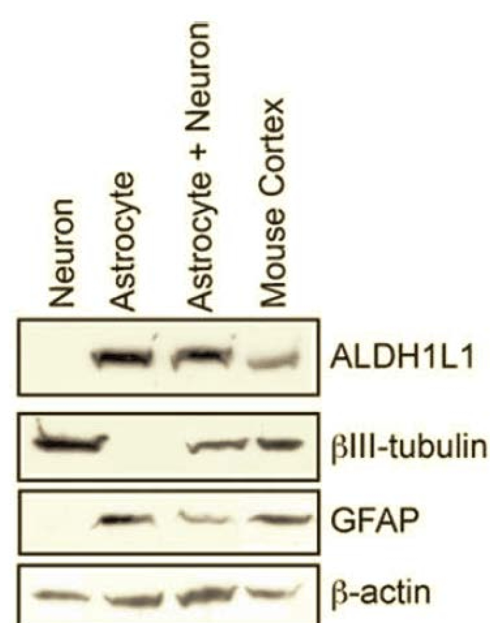
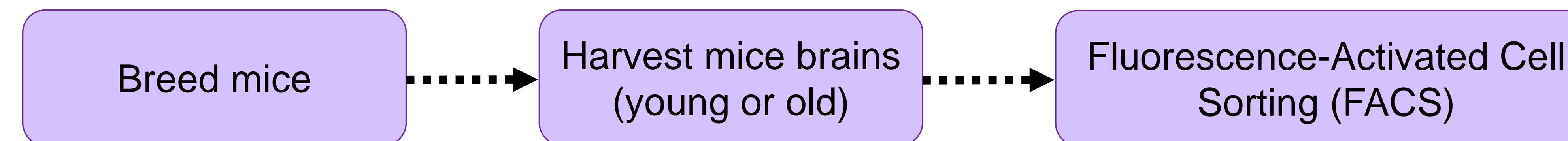


Figure 3. Indication that ALDH1L1 protein is selectively expressed in astrocytes. *Image credit: Yang et al., 2011.*

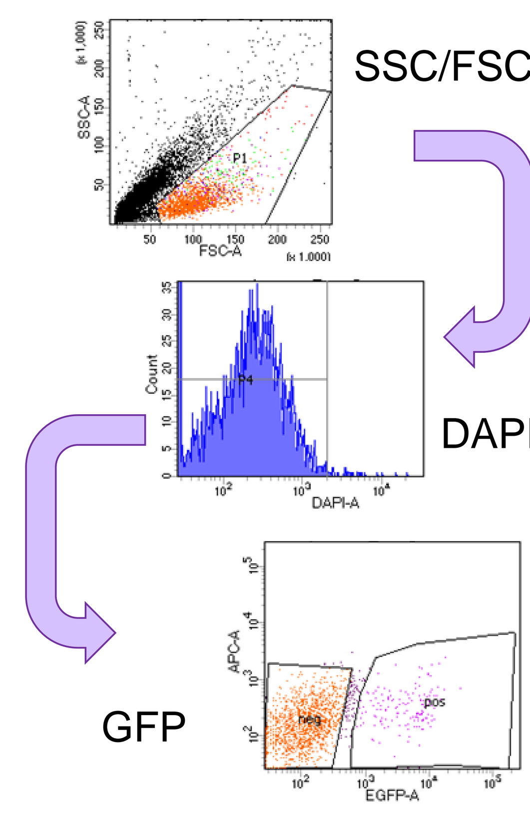
The ALDH1L1+/EGFP+ BAC mouse strain was developed by Rothstein to mark the selective expression of AldH1L1 that occurs in astrocytes with EGFP (Yang *et al.*, 2011). Thus, astrocytes may be sorted from other brain cells using FACS by gating on EGFP+ cells.

Methods

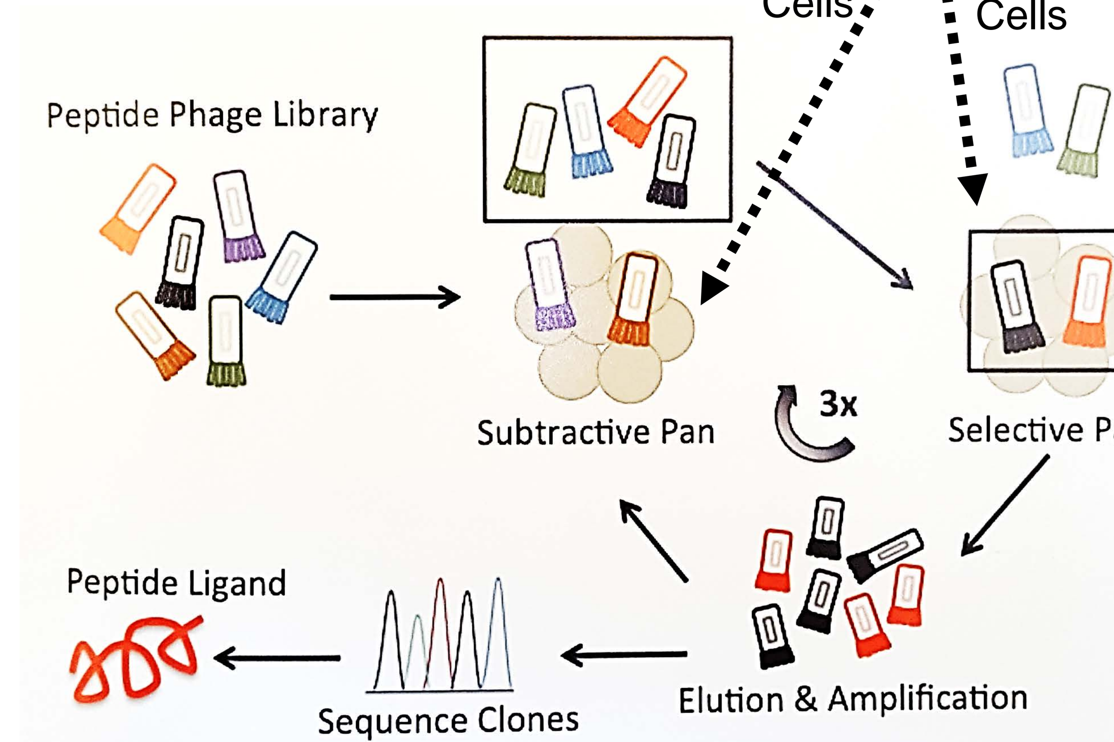
Astrocyte Purification



FACS Gating Strategy



Phage Display



Results

Sort	# of Mice	Mice Age	ALDH1L1/GFP + Cells	Negative Sorted Cells	Efficiency
1	5	P2	11,334	124,767	~97%
2a/2b	6	P8	1,947570	6,000,000	100/94%
3	4	P7	~750,000	3,000,000	97%

Table 1. Results from FACS for Sorts 1 through 3. Sorting P2 mouse brains resulted in very low yield and viability. After switching to ~P7 mice, over 1 million GFP+ astrocytes were routinely isolated from each sort.

Attempt	Pan Type	# of Cells GFP- : GFP+	Unamplified Titer (PFU)	% Wildtype Contam.	Amplified Titer (PFU)	% Wildtype Contam.
1	Enrichment	0 : 1E+06	3.21E+07	0	5.44E+13	0
	Subtractive	5E+05 : 1E+06	4.32E+05	0	N/A	>50
2	Subtractive	1E+06 : 6E+05	1.79E+06	0	1.25E+13	50
3	Enrichment	0 : 1E+06	1.77E+08	0	3.43E+12	0

Table 2. Results from titers and amplification for Attempts 1 through 3. Starting library PFU = 2E+11

Discussion and Conclusions

We have established a protocol for the isolation of live primary astrocytes and completed several rounds of subtractive phage display panning with these cells to identify peptides that bind specifically to astrocytes. Contamination of the library by wildtype phages both 1) decreases library titering accuracy and 2) interferes with the binding of library phage to our target. Once introduced into the amplification process, wildtype phages have a selective replication advantage due to their genetic simplicity compared to the library phage. In our work, we sought to decrease the risk of contamination in three ways: 1) sterilization by Clidox, not just autoclave 2) an increase in selective pressure imposed by altering cell numbers in binding experiments and 3) starting over with new library materials. Although it is impossible to know which method is most effective, following through with all three methods will increase the likelihood of limiting contamination.

Future Work

To further our search for astrocyte-specific targeting ligands, we plan to:

- Complete one more subtractive pan, without the amplification step, for Attempt 2.
- Continue with up to three more subtractive pans for Attempt 3.
- Sequence the peptide inserts of phage obtained through successive panning with next-generation sequencing.
- Maybe: Switch to the alternate evolutionary ligand selection process of using aptamers.

Acknowledgments

- Lisa Peterson, Greg Diggs-Yang, and contributors to GenOM ALVA
- University of Washington GenOM ALVA (NIH 5R25HG007153-04)
- Donations from Dr. Anne Dinning and Dr. Michael Wolf

References

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