Targeting Astrocyte Phenotypic Switch after Neural Injury

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Statement of Purpose: Over 1.7 million persons sustain a traumatic brain injury (TBI) in the U.S. alone. Current diagnostic techniques for TBI are excellent in detecting gross morphological alterations; however, they do little to detect the immediate molecular/cellular alterations. Therefore, there is a critical unmet need to develop targeting motifs that are sensitive to the heterogeneous molecular pathologies associated with TBI. The mechanical insult from TBI initiates immediate cellular death (i.e. primary injury) and stimulates a broad range of complex deleterious signaling cascades (i.e. secondary injury) including a phenotypic switch in astrocytes from basal to reactive phenotypes. This nuanced astrocyte phenotypic alteration is an ideal candidate to develop the next generation molecular targeting strategies diagnostic and therapeutic applications. In this study, we employed phage display biopanning with three single chain variable fragment (scFv) libraries (Tomlinson I+J, Domain antibody library) against viable basal and reactive astrocyte cultures (*in vitro*). Here, we report identification and characterization of novel scFvs that (1) distinguish viable unfixed astrocytes over other cell types and (2) preferentially bind reactive astrocytes over basal astrocytes.

Methods: The overall goal was to identify a novel antibody fragment that displays high affinity to reactive astrocytes, presumably through binding to surface protein that is unique to or highly upregulated after the phenotypic switch from basal to reactive astrocytes. Here, primary type-1 astrocytes (rat cortical tissue; P0-P1) were isolated and cultured using standard cell culture methods. Astrogliosis was induced *in vitro* through the application of transforming growth factor-β (TGF-β) [1]. To achieve a specific high affinity scFv, we modified established biopanning phage display protocols to screen against adherent viable astrocytes using three scFv libraries (Tomlinson I+J, Domain antibody library) [2]. Briefly, the biopanning protocol consisted of one round of negative screening against basal adherent basal astrocytes followed by three rounds of active screens against adherent reactive astrocytes. Polymerase chain reactions (PCR) and sequencing enabled monitoring of bacteriophage mutations and/or frame shifts during the screening process. Upon completion of the third positive screen, 96 clones were randomly selected and evaluated for preferential binding to reactive versus basal astrocytes via a modified cell-based enzyme-linked immunosorbent assay (ELISA). The top 10 reactive astrocyte clones from each library were then isolated and amplified to characterize the binding affinity with a more precise concentration dependent ELISA. Results were fitted to a three-parameter non-linear logarithmic equation to compare the half-maximal response (EC50) for each clone.

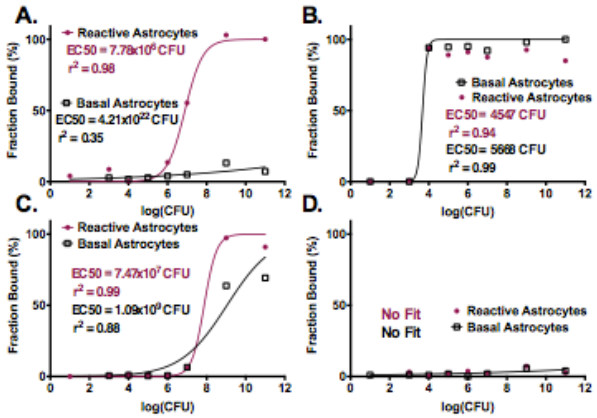

Figure 1 – Concentration dependent binding curves for four scFv clones to basal or reactive astrocytes; clone with affinity to reactive astrocytes (A), clone high affinity to basal and reactive astrocytes (B), moderate affinity clone to basal and reactive astrocytes (C), and negative control clone (D).

Results: Upon completion of the phage biopanning screens and modified ELISAs, we identified one scFv demonstrated affinity and specificity to reactive astrocytes over basal astrocytes (clone F8; **Figure 1A**); multiple scFv clones of interest demonstrated high affinity toward astrocytes regardless of phenotype (**Figure 1B-C**). Moreover, the half-maximal response (EC50) as determined from a non-linear three-parameter logarithmic curve for clone F8 indicated a significant decrease (i.e. higher affinity) in the presence of reactive astrocyte phenotype compared to basal astrocytes (**Figure 1A**). Additionally, corresponding immunohistochemistry with these specific clones support ELISA results (data not shown).

Conclusions: In summary, phage biopanning assays were modified to conduct screens against a complex adherent viable cell target. We identified and characterized scFvs that (1) distinguish viable unfixed astrocytes over other cell types and (2) preferential affinity to reactive astrocytes over basal astrocytes. Such targeting motifs will be critical in developing future diagnostic and therapeutic strategies for neural injury that are sensitive to the injury microenvironment.

Acknowledgements: Authors acknowledge the following funding sources: ASU Startup Funds (S.E.S). We acknowledge Dr. Michael Sierks (ASU) and Dr. Thomas Barker for assistance with phage display.

References:

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