Alterations in Neural Stem Cell Fate Following Focal Ischemia

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Abstract

The purpose of these series of experiments is to observe the fate of neural stem cells in the subventricular zone (SVZ) of adult mouse brain after a middle cerebral artery occlusion (MCAO). Previously, adult neural stem cells (NSC) in the SVZ have been observed to differentiate predominantly into cells with neuronal characteristics. Injury to the brain causes a large number of changes including inflammation and apoptosis, but the reaction of NSC's has been more difficult to characterize because of the transient nature of their response. This study used a tamoxifen-inducible cre-recombinase (Cre-ER^{T2}) expression mouse model system driven by the Cystatin-C promoter to label NSC's in a time specific manner and track their cell fate after injury. Cell fate was determined using established immunohistochemical markers and the fate of NSC's was determined at different time points following a stroke. After the MCAO, the stem cell's differentiation patterns changed from primarily neurogenesis to gliogenesis, specifically producing astrocytes and are observed migrating toward the site of injury. This phenomenon starts 3 days post-injury, peaks at 7 days post-injury, and then diminishes at day 14 post-injury. At 7 days post-injury there is a marked decrease in DCX expressing cells and a concomitant increase in GFAP expressing cells indicating the increase of cells with an astrocyte lineage instead of the predicted neuronal lineage. These results indicate that after a stroke, NSCs within the SVZ regions of the brain undergo a constant change of programmed cell fate, alternating between immature neurons and astrocytes.

Experimental Design

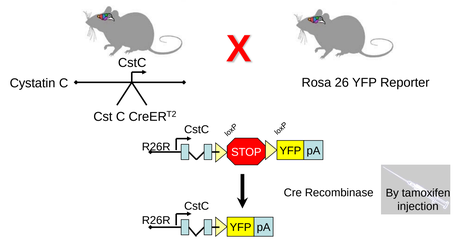

Figure 1. Cystatin C (CstC) Expression

Our aim was to generate a transgenic mouse with a tamoxifen-inducible cre-recombinase (Cre-ER^{T2}) expression system to observe the programmed fate of neural stem cells after a focal ischemia.

Cystatin C was identified as present in NSC's through a genetic screen to uncover novel stem cell specific biomarkers. Following careful validation of cell type specificity, BAC clones expressing 8-10kb of the CstC promoter were identified and used to create a CstC-CreER^{T2} plasmid. Subsequently, transgenic mice were generated by standard recombineering strategy. Several founder lines were carefully screened by crossing to Rosa26-YFP reporter mice. Efficient recombination was seen to occur following systemic treatment with tamoxifen (180 mg/kg for 5 consecutive days), With the Rosa26-YFP reporter, all cells and their progeny that will possess the CstC promoter will be labeled with YFP.

Methods

Middle Cerebral Artery Occlusion[1]

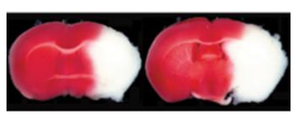

Figure 2. MCAO Representation[2]

Images depict the location of the infarction in relation to the mouse brain. The ischemia affects part of the cerebral cortex as well as most of the striatum. This location is near the lateral ventricle which is part of the subventricular zone (SVZ). In living tissue TTC is enzymatically reduced by dehydrogenases to 1,3,5-triphenylformazan (TPF), which is red in color, while in ischemic areas it remains white due to absence of such enzymatic activity. Therefore, the area of infarction can be identified by its white color due to lack of conversion of TTC to TPF.

For the ischemic stroke model, we used the transient middle cerebral artery occlusion. All middle cerebral artery occlusion were performed by the Neuro-Models Facility. Two adult male and one adult female CstC-CreERT2/Rosa YFP mice were anesthetized with isoflurane and cleaned with betadine at the surgical site. A midline neck incision was made and the soft tissues over the trachea were retracted. The common carotid artery (CCA) was identified and the external carotid artery (ECA) and the internal carotid artery (ICA) were isolated. A monofilament was inserted into the ICA and advanced into the middle cerebral artery (MCA) from the CCA junction for 45 minutes. After the occlusion period, the filament was removed and the incision site was closed. The animals were allowed to recover over heat and buprenophrine was administered. Cerebral blood flow measurements were taken prior to filament insertion, immediately following the middle cerebral artery occlusion (MCAO), after filament removal, and 24 hours following recovery to confirm proper transient occlusion.

Results

Labeling of CstC-CreER^{T2} Cells with Astrocyte Markers in Subventricular Region

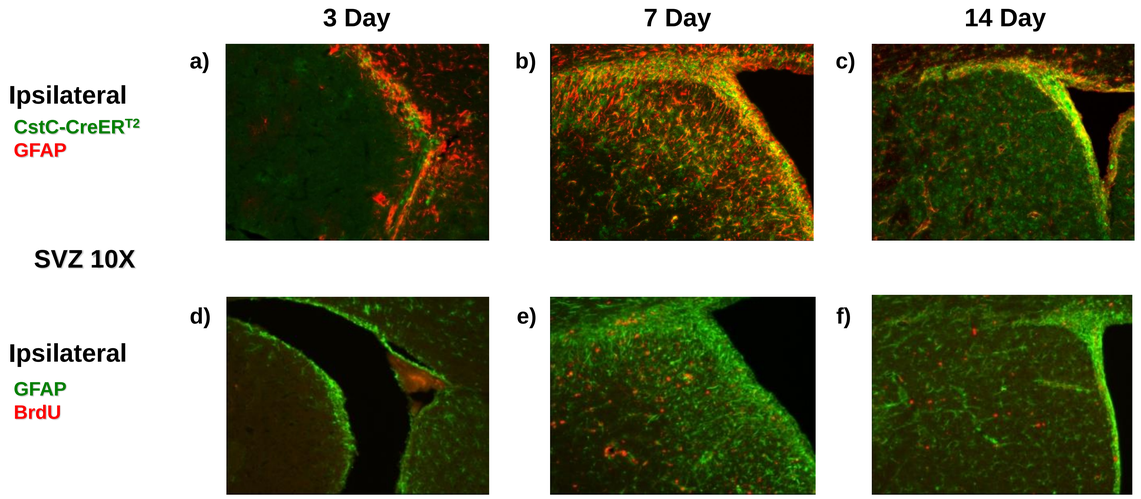

Figure 3. Qualitative Analysis of CstC-CreER^{T2}/Rosa-YFP Mouse Model Stained with GFAP & BrdU

Images depict the time point progression of NSCs in the SVZ of the ischemic brain side that express the GFAP and BrdU markers. Because this is a CreER^{T2} expression system, all cells that possess the CstC gene will stain green. The CstC gene, however, is present in both NSCs and astrocytes. Thus, by further staining with GFAP (a-c), an astrocytic marker, this will differentiate NSCs from astrocytes. Cells that are co-labeled, expressing both GFP and GFAP, are considered to be astrocytes. Cells that are co-labeled (d-f), expressing both GFAP and BrdU, are considered to be newly developed astrocytes that have differentiated from NSCs.

Labeling of CstC-CreER^{T2} Cells with Neuron Markers Subventricular Region

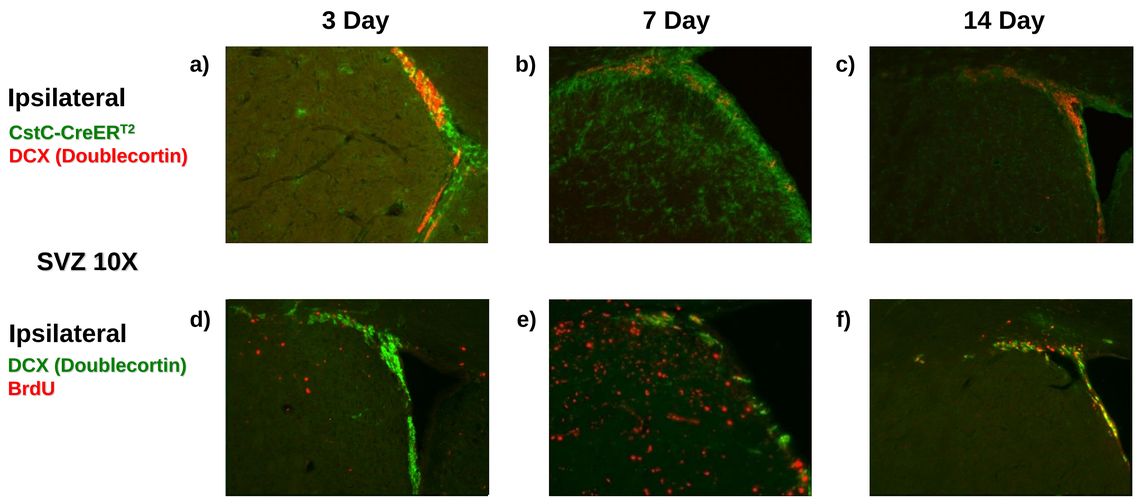

Figure 4. Qualitative Analysis of CstC-CreER^{T2}/Rosa-YFP Mouse Model Stained with DCX & BrdU

Images depict the time point progression of NSCs in the SVZ of the ischemic brain side that express the GFP and DCX markers. Because this is a CreER^{T2} expression system, all cells that possess the CstC gene will stain green. Cells that are co-labeled (a-c), expressing both GFP and DCX, are considered to be NSCs that are becoming immature neurons. Cells that are co-labeled (d-f), expressing both DCX and BrdU, are considered to be newly developed neurons that have differentiated from NSCs.

Results Cont.

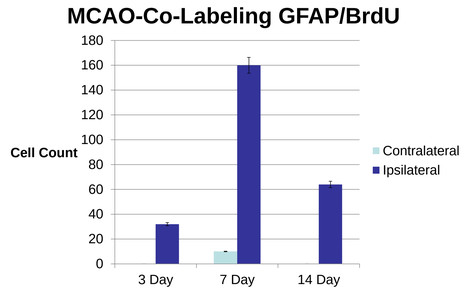

Figure 5. Quantitative Analysis of Growth Rates of Replicating Astrocytes

Growth rates depict the number of CstC-CreER^{T2} cells on either the contralateral (normal) side or ipsilateral (ischemic) side of the brain with a positive signal for both the GFAP and the BrdU antibody staining. Because BrdU provides a more accurate measurement of replication, observing the pattern of co-labeled cells will yield evidence that can validate the switched cell fate after an ischemia. Of a total of 32 sections, every 8th section was stained and counted. Values of each individual section were multiplied by 4 and added together to get representative value for the subventricular zone. Error bars represents the standard deviations for each average cell count. The graph displays specifically only the number of astrocytes within the SVZ undergoing replication for both the normal and ischemic side of the mice brain.

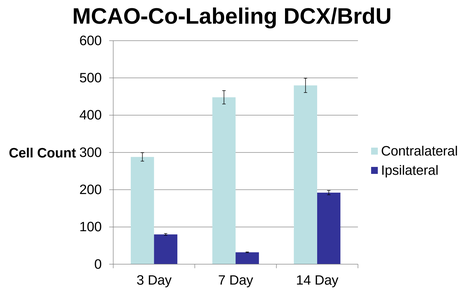

Figure 6. Quantitative Analysis of Growth Rates of Replicating Neurons

Growth rates depict the number of CstC-CreER^{T2} cells on either the contralateral (normal) side or ipsilateral (ischemic) side of the brain with a positive signal for both the DCX and the BrdU antibody staining. Because BrdU provides a more accurate measurement of replication, observing the pattern of co-labeled cells will yield evidence that can validate the switched cell fate after an ischemia. Of a total of 32 sections, every 8th section was stained and counted. Values of each individual section were multiplied by 4 and added together to get representative value for the subventricular zone. Error bars represents the standard deviations for each average cell count. The graph displays specifically only the number of neurons within the SVZ undergoing replication for both the normal and ischemic side of the mice brain.

Conclusions

- Some of the neural stem cells change their programmed fate from neurons to astrocytes.
- The increase in BrdU positive cells 7 days after an ischemia on the ipsilateral side demonstrates an increase of stem cell replication in response to injury.
- The increased number of co-labeled GFAP/BrdU cells indicates an increased proliferation of transient amplifying cells committed to the astrocyte fate.
- The decreased number of co-labeled DCX/BrdU cells after injury reinforces the observation that injury induces a switch in stem cell differentiation.

References

1. Macrae, M. (n.d.). Focal Ischemia Models: Middle Cerebral Artery Occlusion. In *Rodent Model of Stroke* (Vol. 47, pp. 41-53)

2. Chiang T., Messing R.O., Chou W. (2011). Mouse Model of Middle Cerebral Artery Occlusion. JoVE. 48. http://www.jove.com/details.php?id=2761, doi: 10.3791/2761