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Notch1-STAT3-ETBR signaling in brain injury and cancer

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Based on their relative distance from sites of CNS injury or disease and the severity of insult, astrocytes and other glial cells characteristically become “reactive”. Reactive astrocytes are phenotypically different from mature protoplasmic or fibrous astrocytes that reside in normal, healthy tissue. During the process of reactive astrogliosis, mature astrocytes undergo dramatic changes in gene and protein expression that beget changes in morphology (hypertrophy) and cell cycle status (Fig 1). Proliferating reactive astrocytes in close proximity to damaged/dying cells carry out key functions that include neuroprotection, preservation/re-establishment of the blood-brain barrier (BBB), regulation of immune cell responses, and glial scar formation [1]. Importantly, mechanistic understanding and mapping of the signaling network that controls reactive astrogliosis has great potential to elucidate therapeutic targets and inform the development of new treatments to promote recovery and improve outcomes in patients with CNS injury or disease.

Multiple proteins/peptides commonly released during CNS injury have been shown to stimulate reactive astrocyte proliferation such as epidermal growth factor (EGF), fibroblast growth factor (FGF), and endothelin (ET-1). Other cytokines/growth factors, molecules, and conditions known to trigger reactive astrogliosis include (but are not limited to): IL-1, IL-6, IL-10, LIF, CNTF, BMP, TGF- β , TNF- α , INF- γ , lipopolysaccharide, glutamate, adenosine triphosphate, nitric oxide, reactive oxygen species, and hypoxia and glucose deprivation during tissue ischemia [1]. Although numerous signals released or produced during CNS injury have been shown to stimulate reactive astrocytes in culture, the complex signaling network that regulates reactive astrocyte proliferation and function(s) *in vivo* remains poorly understood.

Reactive astrocytes commonly exhibit increased expression of several intermediate filament proteins such as Glial Fibrillary Acidic Protein (GFAP), Nestin, and RC2, a specialized isoform of Nestin with post-translational modifications (Fig 1). Previously, our group demonstrated Notch1 signaling was required for the proliferation of reactive astrocytes after stroke. Using an inducible transgenic mouse model with Cre-loxP-mediated gene excision (GFAP-CreER-Notch1-cKO), we reported that conditional knockout (cKO) of Notch1 in

reactive astrocytes significantly reduced the number of RC2-positive cells immediately adjacent to the infarct core and increased immune cell invasion after stroke [2].

Recently, we demonstrated that the RC2 antigen marks specifically the subpopulation of proliferating reactive astrocytes after stroke and traumatic brain injury and reported a method to prospectively isolate the subpopulation of proliferating reactive astrocytes from injured CNS tissue based on expression of the glutamate-aspartate transporter (Glast) and Jagged1, a Notch1 ligand [3]. In the peri-infarct area after stroke, RC2-positive reactive astrocytes expressed high levels of ETB_R, prompting us to investigate whether Notch1 signaling interacted with ETB_R. We found that Jagged1/Notch1 signaling increased ETB_R levels in an indirect manner, by stabilizing phosphorylated STAT3 (p-STAT3), a transcriptional activator for *EDNRB* (ETB_R gene). Subsequent experiments with GFAP-CreER-ETB_R-cKO mice demonstrated that ETB_R was necessary for reactive astrocyte proliferation after stroke. Notably, the deficit observed in GFAP-CreER-ETB_R-cKO mice closely resembled that of GFAP-CreER-Notch1-cKO mice. Notch1-STAT3-ETB_R signaling may affect numerous cell types that proliferate during development, tissue repair, and cancer. In addition to primary adult astrocytes, we observed that Notch1-STAT3-ETB_R signaling regulated proliferation and/or survival of primary neonatal astrocytes as well as human tumor cell lines derived from kidney (HEK 293) or brain (U87; glioma, astrocytoma)[3].

Whereas we found that Jagged1/Notch1 signaling promoted increased ETB_R levels in postnatal astrocytes and adult reactive astrocytes, ET-1 was recently shown to induce Jagged1 expression in cultured astrocytes and to alter Notch signaling *in vivo* during the first week after lysocleithin-induced focal demyelination [4]. In the same injury model, reactive astrogliosis was previously shown to result in ET-1 signaling through ETB_R, ERK and JNK pathways, and activation of the transcription factor c-jun [5]. Importantly, c-jun can bind to cis-acting AP-1 DNA elements in the *Jag1* promoter to drive Jagged1 expression [6]. In light of these observations, our recent data suggest a feed-forward loop whereby ET-1 signaling through ETB_R increases Jagged1 levels, which then can activate Notch1 signaling in adjacent astrocytes. Notch1 signaling increases the expression of ETB_R by stabilizing p-STAT3, and in turn enhances expression of Jagged1 by amplifying ET-1 signaling through ETB_R. Accordingly, we expect that Jagged1/Notch1, STAT3, and ET-1/ETB_R signaling all contribute to driving reactive astrocyte proliferation after CNS injury through this feed-forward loop. Similar feed-forward signaling loops promoted by STAT3 may be a common driver for rapid cell proliferation after tissue injury and during cancer. Feed-forward signaling through STAT3 is reported to regulate cell proliferation in tumors and transformed cells from several tissue types as well as those of the blood [7–9].

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Fig 1. Sub-types of reactive astrocytes in the cerebral cortex 3 days after stroke

(A) Distal to the stroke infarct, GLAST⁺ protoplasmic astrocytes within uninjured parenchymal tissue express undetectable or low levels of Jagged-1 and ETB_R and are negative for GFAP. (B) Towards the outer edge of the peri-infarct area, GLAST⁺ reactive astrocytes express low levels of Jagged-1, ETB_R and GFAP, but do not undergo cell division. (C) Located immediately adjacent to the infarct core, proliferating GLAST⁺ reactive astrocytes express GFAP, Nestin, and the RC2 antigen. Notably, sub-type (C) is significantly depleted after conditional knockout of Notch1 or ETB_R in GFAP-positive reactive astrocytes

in transgenic mice with stroke. Note that all three astrocyte subtypes express cytokine receptors such as gp130, and are thus sensitive to inflammatory mediators such as IL-6.

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