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STAT3-regulated RhoA drives reactive astrocyte dynamics

Francois Renault-Mihara and Hideyuki Okano

Department of Physiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo, Japan

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Signal transducer and activator of transcription-3 (STAT3), a prominent member of the STAT family of transcription factors, is a core component of the JAK-STAT3 signal transduction pathway. Early studies have indicated the critical role of STAT3 in astrocytes, a subtype of glial cells (*i.e.*, non-neuronal cells) that are present in the central nervous system. STAT3 is a key factor in astrocyte genesis during embryonic development and is crucial for inducing reactive astrocytes in a variety of diseases and injuries. A previous study from our laboratory showed that STAT3 signaling in reactive astrocytes is required for proper glial scar formation after spinal cord injury (SCI).[1] Specific ablation of STAT3 in reactive astrocytes results in defective glial scars, widespread infiltration of leukocytes, enhanced demyelination, and increased neuronal loss after contusive SCI. Conditional deletion of STAT3 in reactive astrocytes (STAT3-cKO) impairs migration after *in vitro* scratch injury,[1] which suggests that the defective scarring observed in *Nestin*-Cre;*Stat3*$^{fl/fl}$ mice after SCI is due to the defective re-organization of reactive astrocytes. In support of this hypothesis, seclusion of various non-neural cells that invade the lesion core has been shown to depend on the STAT3-dependent reorientation of astrocytic processes.[2] Although multiple studies have explored the mechanism of STAT3 regulation of cell migration in various cell types, the downstream effectors of STAT3 in reactive astrocyte dynamics remain unknown.

Our recent study examined the mechanisms of STAT3 regulation of reactive astrocyte dynamics *in vitro* and glial scar formation *in vivo*.[3] We first examined several known effectors of STAT3 in cell dynamics/migration, and we observed reduced levels of matrix metallopeptidase-2 (MMP2), a known direct transcriptional target of STAT3, in the conditioned medium of STAT3-cKO astrocytes. In the context of SCI, this observation is relevant because MMP-2 promotes functional recovery after SCI by regulating glial scar formation.[4] Notably, the previously reported regulation of microtubule dynamics through STAT3 interaction with stathmin was not observed in astrocytes in our study, as the microtubule cytoskeleton of STAT3-cKO astrocytes was unaltered. Further examination of the mechanisms through which STAT3 regulates reactive astrocyte dynamics revealed a critical role for the small GTPase RhoA, a key regulator of actin dynamics. We observed that RhoA is constitutively elevated in STAT3-cKO astrocytes *in vitro* and that elevated RhoA activity increases acto-myosin tonus, thereby impairing astrocyte migration. Acto-myosin-generated tensions are known to control the molecular kinetics of focal adhesion complexes (FAs). Accordingly, time-lapse recordings of migrating astrocytes showed that STAT3 is critical to the dynamics of FAs. STAT3-cKO astrocytes exhibit slower FA dynamics, and this appears to be the result of impaired disassembly of FAs due to the insensitivity of the STAT3-cKO astrocytes to Src inhibition. Although Src and FAK are known to be involved in FA disassembly, they both exhibit normal levels of expression and phosphorylation in STAT3-cKO astrocytes. Although RhoA is known to modify astrocyte morphology,[5] the observed upregulation of RhoA in STAT3-cKO astrocytes was unexpected. A previous study of mouse embryonic fibroblasts showed that loss of STAT3 expression resulted in increased Rac1 activity[6] and subsequent antagonization of RhoA; this finding highlights the unique output of STAT3 in reactive astrocytes. Furthermore, we showed that RhoA's normal activity is necessary to corral leukocytes by reactive astrocytes *in vitro*. This process is thought to mediate the seclusion of infiltrating meningeal fibroblasts and leukocytes within the lesion center after SCI and to be necessary for the neuroprotective role of scar-forming reactive astrocytes.[2] Inhibition of RhoA by STAT3 seems to involve Ezrin, which is known to down-regulate RhoA activity in other cells. However, how the loss of STAT3 results in reduced phosphorylation of Ezrin at threonine residues is unclear.

Additional experiments showed that phosphatase and tensin homolog (PTEN) is another target of STAT3 signaling that contributes to the dynamics of reactive astrocytes. We found that PTEN is up-regulated in reactive astrocytes lacking STAT3 and that genetic reduction of PTEN levels rescues FA dynamics

CONTACT Francois Renault-Mihara frenaultpro@yahoo.fr; Hideyuki Okano hidokano@keio.jp Department of Physiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.

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and glial scar formation. This observation is consistent with those of previous studies showing that PTEN negatively regulates astrocyte migration and prevents glial scar formation. PTEN is known to be extracellularly released in exosomes and transferred to neighboring cells,[7] and reduction of PTEN levels is known to exert both neuroprotective effects and regenerative action on neuronal axons; therefore, STAT3-mediated repression of PTEN in reactive astrocytes likely has beneficial effects on neighboring neurons.

Our study identified an exciting novel downstream effector of STAT3 signaling, and this finding raises several questions. How precisely does STAT3 regulate the activation of RhoA in astrocytes? What are the consequences of RhoA regulation by STAT3 in astrocytes with respect to astrocyte morphology and the physiological interactions between astrocytes and neurons? We hypothesize that this STAT3/RhoA axis may impact key functions of normal astrocytes, such as glutamate clearance, potassium homeostasis and modulation of synaptic transmission and plasticity. Finally, to what extent can these results be generalized to other cell types in other contexts, such as cancer and immunity? These should be clarified in the future investigations.

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Disclosure of potential conflicts of interest

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ORCID

Francois Renault-Mihara http://orcid.org/0000-0002-7105-7317
Hideyuki Okano http://orcid.org/0000-0001-7482-5935

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