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THE COMPLEX STATES OF ASTROCYTE REACTIVITY: HOW ARE THEY CONTROLLED BY THE JAK–STAT3 PATHWAY?

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Abstract—Astrocytes play multiple important roles in brain physiology. In pathological conditions, they become reactive, which is characterized by morphological changes and upregulation of intermediate filament proteins. Besides these descriptive hallmarks, astrocyte reactivity involves significant transcriptional and functional changes that are far from being fully understood. Most importantly, astrocyte reactivity seems to encompass multiple states, each having a specific influence on surrounding cells and disease progression. These diverse functional states of reactivity must be regulated by subtle signaling networks. Many signaling cascades have been associated with astrocyte reactivity, but among them, the JAK–STAT3 pathway is emerging as a central regulator. In this review, we aim (i) to show that the JAK–STAT3 pathway plays a key role in the control of astrocyte reactivity, (ii) to illustrate that STAT3 is a pleiotropic molecule operating multiple functions in reactive astrocytes, and (iii) to suggest that each specific functional state of reactivity is governed by complex molecular interactions within astrocytes, which converge on STAT3. More research is needed to precisely identify the signaling networks controlling the diverse states of astrocyte reactivity. Only then, we will be able to precisely delineate the therapeutic potential of reactive astrocytes in each neurological disease context.

Key words: reactive astrocytes, STAT3, JAK–STAT pathway, neurological diseases, signaling cascades.

Contents

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Abbreviations: AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; CNTF, ciliary neurotrophic factor; Cx43, connexin 43; DN, dominant negative; EGF, epidermal growth factor; ETC, electron transport chain; GFAP, glial fibrillary acidic protein; GPCR, G-protein-coupled receptor; HD, Huntington's disease; HDAC, histone deacetylase; IL, interleukin; JAK, janus kinase; KO, knock out; Lcn2, lipocalin 2; LPS, lipopolysaccharide; Lys, lysine; MAPK, mitogen-activated protein kinase; MCAO, middle cerebral artery occlusion; MMP, matrix metallo-proteinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MS, multiple sclerosis; mSTAT3, mitochondrial STAT3; MnSOD, manganese superoxide dismutase; ND, neurodegenerative diseases; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PD, Parkinson's disease; PIAS, protein inhibitor of activated STAT; PKR, protein kinase R; PTM, post-translational modifications; ROS, reactive oxygen species; SCI, spinal cord injury; Ser, serine; Shh, Sonic Hedgehog; SOCS, suppressor of cytokine signaling; SRE, STAT3-responsive element; STAT, signal transducer and activator of transcription; TBI, traumatic brain injury; TSP1, thrombospondin-1; Tyr, tyrosine; UCP, uncoupling protein.

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INTRODUCTION

In response to the multiple pathological conditions that affect the central nervous system (CNS), astrocytes become reactive. This response develops after acute injuries such as ischemia, traumatic brain injury (TBI), spinal cord injury (SCI) or infection, as well as under progressive conditions like neurodegenerative diseases (ND) or multiple sclerosis (MS). Astrocyte reactivity was initially characterized by morphological changes (hypertrophy of soma and processes) and by the upregulation of intermediate filament proteins such as glial fibrillary acidic protein (GFAP) or vimentin. Besides these two hallmarks, astrocyte reactivity involves multiple transcriptional and functional changes that are still being elucidated (Burda and Sofroniew, 2014; Pekny and Pekna, 2014; Ben Haim et al., 2015a). Importantly, astrocyte reactivity is now recognized as a heterogeneous response resulting in various functional states depending on the disease context. In fact, it is important to note that reactivity is not the only change observed in astrocytes during diseases. For example, astrocytes may be dystrophic in the brain of patients with schizophrenia or even degenerate following encephalopathies. They may directly be hit by the disease and dysfunction, like in Alexander's disease, which is caused by mutations in the *gfap* gene (Verkhratsky et al., 2015; Pekny et al., 2016).

Given the multiple roles operated by astrocytes in physiological conditions (Parpura et al., 2012), the functional changes occurring with reactivity could have major consequences on surrounding cells like neurons or microglial cells and influence disease progression. Therefore, it is crucial to unravel the signaling cascades controlling the specific states of astrocyte reactivity.

Multiple pathways are associated with astrocyte reactivity (Buffo et al., 2010; Kang and Hebert, 2011; Ben Haim et al., 2015a). Among them, the janus kinase-signal transducer and activator of transcription 3 (JAK–STAT3) pathway seems to play a central role that we will cover in this review.

We will focus on the roles of STAT3 in astrocytes during various brain diseases, but we will also describe data from other cell types in the CNS or peripheral organs, when they give insight into the functions of the JAK–STAT3 pathway. Indeed, seminal discoveries on this cascade were made in cell cultures in the fields of Immunology and Oncology. Although very potent, *in vitro* studies have serious limitations when applied to the brain, because of the significant phenotypic changes occurring when brain cells are isolated in a dish. This is especially true for primary astrocytes that tend to become reactive without stimulation (Ben Haim et al., 2015a). In this review, we will thus present *in vivo* studies whenever possible, and landmark *in vitro* studies to illustrate the complex roles played by STAT3 in the control of astrocyte reactivity. We will (i) show that the JAK–STAT3 pathway plays a key role in the control of astrocyte reactivity, (ii) illustrate that STAT3 is a pleiotropic molecule operating multiple functions in reactive astrocytes and (iii) propose that each specific functional state of reactivity is governed by complex molecular interactions within astrocytes, which converge on STAT3.

THE JAK–STAT3 PATHWAY

A linear, canonical JAK–STAT3 pathway from the membrane to the nucleus

The JAK–STAT pathway is a ubiquitous, evolutionarily conserved signaling cascade, present in various species from *Dictyostelium* and *Drosophila* to mammals (Decker and Kovarik, 2000). It was discovered more than twenty years ago, as the cascade mediating interferon effects (Darnell et al., 1994; Stark and Darnell, 2012). There are four JAKs (JAK1-3, and TYK2) and seven STATs (STAT1-4, 5A, 5B, and 6) in mammals (Darnell, 1997). STAT3 was sequenced and cloned in 1994 (Akira et al., 1994; Zhong et al., 1994b). STAT3 is well expressed in the brain (Zhong et al., 1994a) and has been the most extensively STAT studied in the context of astrocyte reactivity.

The canonical JAK–STAT3 pathway is activated by the binding of polypeptides such as cytokines, hormones or growth factors to their multimeric receptor (Mertens and Darnell, 2007, Fig. 1). Conformational changes on the intracellular tail of the receptor bring the kinase domains of two JAKs in apposition (Brooks et al., 2014). JAKs are receptor-associated tyrosine (Tyr) kinases that phosphorylate each other and the receptor on several residues. The latent transcription factor STAT3 is then recruited to the phosphorylated receptor through its Src homology 2 (SH2) domain and is transphosphorylated by JAK on Tyr705 (Lim and Cao, 2006). Phospho-STAT3 proteins dimerize and accumulate in the nucleus. There, dimers of phospho-STAT3 bind specific sequences called STAT3-responsive elements (SRE) in the promoter of target genes and induce their transcription (Shuai et al., 1993; Darnell, 1997). These transcriptional changes impact cell growth, proliferation, differentiation and survival. This pathway is particularly important during development and immune responses, and its dysregulation is involved in cancer and immune diseases (see, Yu et al., 2009; O'Shea et al., 2013, for a complete review, as this will not be developed here). Activation of the JAK–STAT3 pathway increases the expression of several elements of the pathway, including *stat3* itself, which promotes a positive feedback loop (Hutchins et al., 2013).

Additional branching points on the pathway increase the complexity of STAT3 signaling cascades

Besides the linear "canonical" pathway, STAT3 is connected to alternative signaling cascades within the cell (Fig. 1).

First, some G-protein-coupled receptors (GPCRs), which are seven-transmembrane domain receptors for growth factors or purines, may be coupled to JAKs and phosphorylate STAT3 (Mertens and Darnell, 2007). Alternatively, STAT3 can be phosphorylated on Tyr705 by other upstream kinases than JAKs (Fig. 1). They include receptors with an intrinsic Tyr kinase activity, like the receptor for epidermal growth factor (EGF), and non-receptor Tyr kinases, which are usually cytoplasmic and of viral origin, such as v-src (Mertens and Darnell, 2007).

In addition, STAT3 can be phosphorylated on Serine 727 (Ser727) by various Ser kinases, especially by mitogen-activated protein kinases (MAPK) (Wen et al., 1995; Decker and Kovarik, 2000). Depending on the