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Research Report

VEGF overexpression enhances the accumulation of phospho-S292 MeCP2 in reactive astrocytes in the adult rat striatum following cerebral ischemia

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ABSTRACT

Purpose: Astrocytes can be reactivated after cerebral ischemia by expressing nestin and other characteristic markers of neural stem cells (NSCs). However, the epigenetic features of reactive astrocytes are not well known. Methyl-CpG-binding protein 2 (MeCP2) is a vital transcriptional modulator in brain development. Although the expression and function of some phosphorylated MeCP2 isoforms have been clarified, phospho-serine 292 (pS292) MeCP2 has not yet drawn much attention. In this study, we used western blot analysis and immunohistochemical and immunofluorescent staining to reveal the expressive features of pS292 MeCP2 and MeCP2 in the adult rat striatum following transient middle cerebral artery occlusion (MCAO).

Results: We first discovered that the ischemia-induced expression of cytoplasmic pS292 MeCP2 is primarily accumulated in nestin-positive reactive astrocytes in the stroke-injured striatum. Moreover, the enhancement of astrocytic pS292 MeCP2 was correlated with the augmentation of VEGF in astrocytes, as determined by the substantial co-localization of pS292 MeCP2 and VEGF after stroke. Finally, the exogenous overproduction of VEGF further promoted the expression of pS292 MeCP2 in reactive astrocytes, and this effect was accompanied by a marked increase in reactive astrocytes. On the contrary, MeCP2 was predominantly expressed in the neuronal nucleus, and the level of this protein was not significantly altered after ischemic injury and VEGF overproduction.

Abbreviations: VEGF, vascular endothelial growth factor; MeCP2, methyl-CpG-binding protein 2; pS292, phospho-serine 292; NSCs, neural stem cells; BBB, blood–brain barrier; MCAO, middle cerebral artery occlusion; RTT, Rett syndrome; PPTase, alkaline phosphatase; phVEGF, pEGFP-N1-VEGF plasmid; pEGFP, pEGFP-N1 empty plasmid; PBS, phosphate-buffer solution; DAB, diaminobenzidine; SEM, standard error of the means; ANOVA, analysis of variance

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Conclusion: Our data provide the first demonstration that overexpression of VEGF enhances the accumulation of pS292 MeCP2 in reactive astrocytes in the ischemic-injured rat striatum, implicating a pS292 MeCP2-related epigenetic role of exogenous VEGF in reactive astrocytes following cerebral ischemia.

1. Introduction

Ischemic stroke caused by a sudden disruption of blood flow in the brain leads to acute neural cell death and a series of neurological deficits. However, effective clinical therapies remain limited and are unable to be applied to a wide range of patients because of their narrow therapeutic window. On this basis, extensive research efforts have been devoted to the activation of endogenous neural stem cells (NSCs), which is considered a potential avenue for the promotion of neuroprotection and restoration of neural networks following stroke (Kalladka and Muir, 2014). Interestingly, astrocytes are reactivated after cerebral ischemia through the expression of nestin, a marker for NSCs. Recently, increasing evidence has demonstrated that nestin-expressive reactive astrocytes acquire stem cell properties after injury and can further differentiate into neurons *in vitro via* transcription factor overexpression (Berninger et al., 2007; Buffo et al., 2008), indicating that reactive astrocytes may function as a source of endogenous multipotent cells under pathological conditions. Therefore, it will be of great value to investigate the characteristics of these ischemia-induced reactive astrocytes in the brain.

Emerging epigenetic mechanisms have recently been shown to serve as intrinsic reprogramming factors to modulate almost every aspect of NSC development, including self-renewal, proliferation, neurogenesis, gliogenesis, cell migration, synaptic plasticity, and neural network integration (Qureshi and Mehler, 2010). Therefore, the involvement of DNA methylation in stroke has been examined in several studies. Endres et al. (2000, 2001) reported that the incorporation of a methyl group into the 5′-cytosine of DNA increased after mild ischemia and that a reduced level of DNA methyltransferase 1 (Dnmt1) in post-mitotic neurons protected from ischemic brain injury by decreasing the infarct volume and increasing the density of neuron-like viable cells, indicating an important role for methylated DNA in stoke-injured brain. Notably, methyl-CpG-binding protein 2 (MeCP2), a member of the methyl-CpG-binding protein family, can bind to the methylated CpGs in the promoters of target genes to regulate transcription (Chahrour et al., 2008). MeCP2 is abundant in mammal brains and primarily expressed in mature post-migratory neurons (Jung et al., 2003). Recently, there is increasing evidence for the critical role of MeCP2 in astrocytes, showing that MeCP2 deficiency in glia could affect the development of neurons, and the restoration of MeCP2 in the mutant astrocytes exerted a non-cell autonomous positive effect on mutant neurons *in vivo* (Ballas et al., 2009; Lioy et al., 2011). However, to date, few studies have investigated the function of MeCP2 in stroke-evoked reactive astrocytes, which will offer a novel epigenetic interpretation to the features of reactivated astrocytes in the ischemic brain.

MeCP2 could be phosphorylated at different serine sites to play distinct roles in transcription. The well-documented phospho-S421 (pS421) or phospho-S80 (pS80) modification causes MeCP2 to isolate from or bind with the promoters of target genes to modulate transcription in opposite manners (Tao et al., 2009; Zhou et al., 2006). Moreover, different phosphorylated MeCP2 isoforms can interact with distinct co-factors to initiate disparate transcriptional pathways (Gonzales et al., 2012), suggesting that phosphorylation modifications can directly regulate the function of MeCP2 in the brain. To the best of our knowledge, there remain many other unexamined serine sites predicted for the phosphorylation of MeCP2, such as S292 (Diaz de Leon-Guerrero et al., 2011), the identification of which may assist us in further comprehending the function of MeCP2.

Vascular endothelial growth factor (VEGF) is a neurotrophic factor with biphasic effects in the ischemic brain. Although systematic delivery of VEGF can be detrimental during the acute injury phase by inducing blood–brain barrier (BBB) leakage and brain edema (Ma et al., 2012; Zhang et al., 2000), topical administration of VEGF exerts significant beneficial effect on the ischemic brain *via* reducing infarct size and promoting neurogenesis in the chronic period (Jin et al., 2002; Wang et al., 2007, 2009). In addition, it has been shown that VEGF and its receptors can be expressed in astrocytes, and hypoxia can further upregulate the level of VEGF in reactive astrocytes (Kaur et al., 2006; Shin et al., 2010), indicating an important role of astrocytic VEGF post stroke. Moreover, although plenty of studies have paid great attention to the signal pathways of VEGF, such as Notch, Pten, MEK/ERK and PI3K/AKT (Fournier et al., 2012; Sun et al., 2010), few investigations have focused on the epigenetic mechanisms involved in this process, and the underlying epigenetic-related function of VEGF in reactive astrocytes also remains largely unknown. On this basis, we examined the expressive characteristics of phospho-S292 MeCP2 (pS292 MeCP2) and MeCP2 in the adult rat striatum following cerebral ischemia and VEGF overproduction to provide some preliminary insights into the MeCP2-mediated epigenetic modulation in the stroke-injured brain.

2. Results

2.1. *Ischemic stroke induced the phosphorylation of MeCP2 at S292 in the adult rat striatum following MCAO*

To determine the expressive characteristics of MeCP2 and pS292 MeCP2 in the adult rat striatum after stroke, we extracted the cytoplasmic and nuclear proteins of the sham-operated striatum