Role of 14-3-3 Proteins in Myelination

Information in the central nervous system (CNS) is propagated through axons of nerve cells that are insulated by a layer of white fatty substance known as the myelin sheath. Destruction of myelin sheaths leads to loss of signal transmission between nerve cells. Remyelination of axons by oligodendrocytes, the myelin producing cells of the CNS, reverses the compromised transmission (1). It has been shown that transplanting oligodendrocyte precursor cells (OPCs) in rat models promotes recovery following spinal cord injury (2). In contrast, pathological negative regulation of OPC differentiation results in demyelinating diseases (3). Thus, targeting OPCs may serve as a method to induce remyelination. The Fournier lab identified the 14-3-3 family of adaptor proteins as regulators of oligodendrocyte maturation in vitro. 14-3-3s are comprised of seven different isoforms, β, γ, ε, ζ, η, θ, and σ, which bind specific serine- and threonine-phosphorylated motifs on client proteins (4). These proteins mediate myriad functions, including protection from dephosphorylation, regulation of enzyme activity, formation of ternary complexes, and sequestration of their substrates (5). Dysregulation of 14-3-3s is commonly observed in neurodegenerative disease involving demyelination (6). Hence, regulation of 14-3-3s may provide a way of modulating OPC differentiation. Fusicoccin, a drug that stabilizes the interaction between 14-3-3s and its client proteins, impairs the differentiation of oligodendrocyte marker 4 (O4)-positive OPCs into myelin basic protein (MBP)-positive mature oligodendrocytes in vitro. Therefore, we hypothesized that the inhibition of 14-3-3-client protein interactions promotes the maturation of oligodendrocytes. To investigate this hypothesis, OPCs were isolated from neonatal rat brains and differentiated for 2 days. The maturation of OPCs into oligodendrocytes was assessed by analyzing O4 and MBP expression via immunocytochemistry.

To investigate the inhibition of 14-3-3 function on oligodendrocyte maturation, OPCs were treated with either the 14-3-3 activity inhibitor, BV02, or the control, DMSO, for two days in vitro (Figure A). The percentage of O4- and MBP-positive cells did not differ between the two experimental groups. The area covered by O4-positive cells also did not differ significantly. However, the area covered by MBP-positive cells increased in the BV02 group compared to the control (Figure B). This result suggests that inhibition of 14-3-3 may not have induced the differentiation of OPCs into oligodendrocytes, but it perhaps enhanced the differentiation of OPCs by promoting their growth. To substantiate this finding, difopein, a peptide that antagonizes 14-3-3-client interactions, was transduced into OPCs via nucleofection (Figure D). Cells transduced with difopein exhibited increased MBP area coverage relative to the control WLRL group (Figure C). Interestingly, the morphology of the nucleofected cells was less elaborate compared to cells treated with BV02. Two-way ANOVA was performed on 4 independent experiments (Figure E). Difopein and WLRL transductions did not significantly change the percentage of cell death for both MBP-positive and -negative cells. However, in both transduction groups the MBP-positive cells displayed significantly more cell death than the MBP-negative cells. This indicates that oligodendrocytes are more sensitive to the transduction protocol. We then sought to identify which 14-3-3 isoforms could be biochemically isolated from oligodendrocytes by western blot (Figure F). Expressions of 14-3-3 β, γ, ζ, and ε were detected after both 2 days in vitro, when the cultures are thought to be mostly immature, and 5 days in vitro, when MBP-positive cells start being abundant in culture. This demonstrates the specificity of the 14-3-3 isoforms expressed during the maturation of oligodendrocytes. Furthermore, no significant difference between the levels can be detected between the two time points, suggesting that their expression is constant throughout OPC differentiation.

The inhibition of 14-3-3-client protein interactions enhances the differentiation of OPCs by promoting their growth. 14-3-3 binding motifs are required for the inhibition of forkhead transcription factor FOXO4 binding to its target DNA (7). Furthermore, 14-3-3 proteins restrain protein phosphatase 2 activity with phosphorylation sites of protein kinase B, a serine/threonine-specific protein kinase that interacts with FOXO3 (8). FOXO transcription factors are involved in the regulation of cell cycle, cell death, and cell metabolism (9). Moreover, estrogen receptor α dimerization is negatively regulated by 14-3-3s, resulting in decreased cell proliferation (10). These phenomena may regulate the balance between cell proliferation and growth. 14-3-3 protein-mediated pathways may inhibit the proliferation of cells to allocate energy to cell growth.

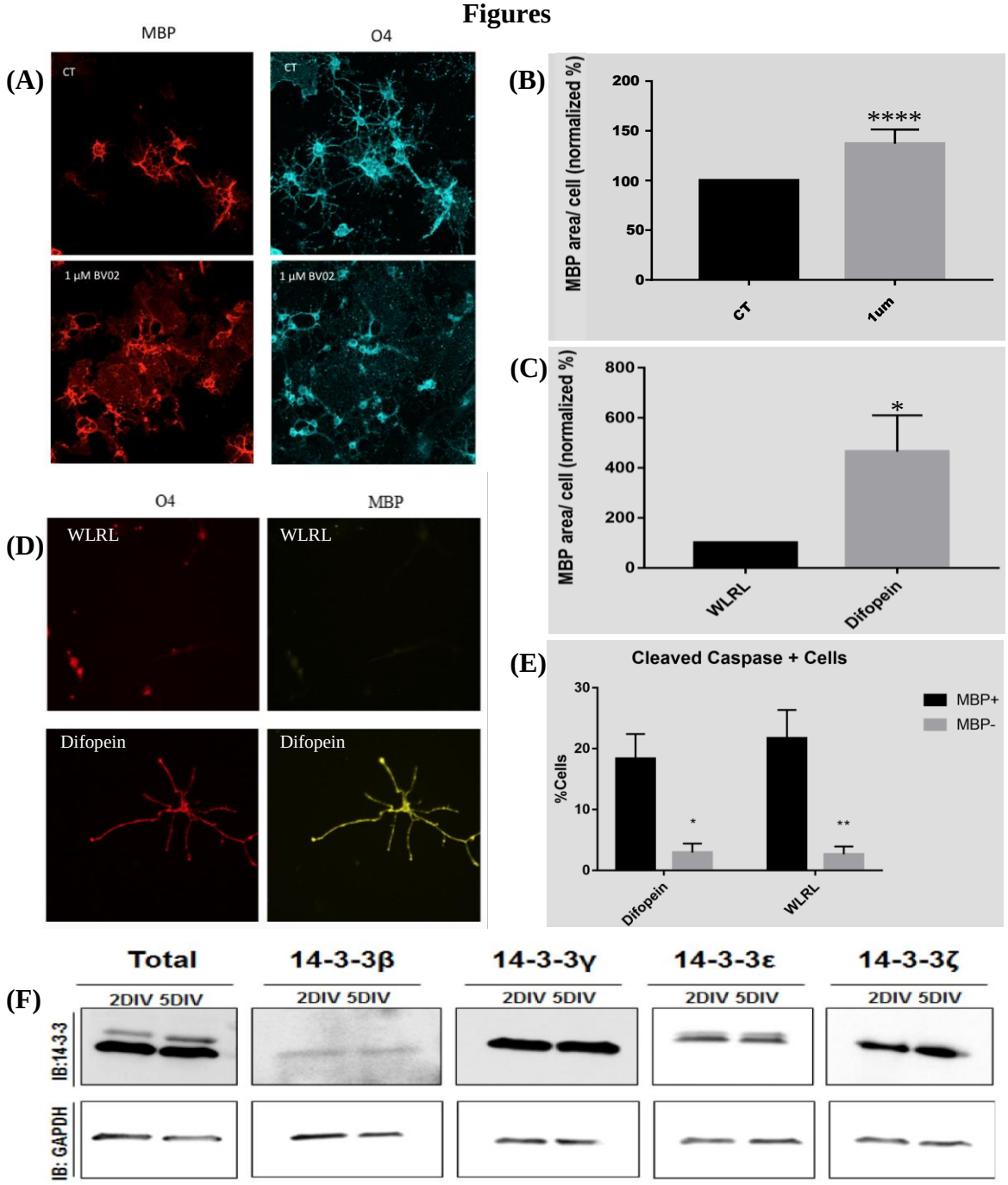
14-3-3 σ has been shown to stabilize FOXO1, which in turn regulates B cell homeostasis (11). FOXO1 is critical for B cell survival (12). B cells could secrete antibodies against myelin (13). B cells could further exacerbate demyelination by secreting proinflammatory cytokines that induce T cells to attack myelin sheaths (14). This may provide a link between the immune system and the CNS during multiple sclerosis. The inhibition of 14-3-3 activity may lead to enhanced myelination by oligodendrocytes and compromised B cell survival due to destabilized FOXO1.

14-3-3s modulate the switch from sonic hedgehog-mediated axon attraction to repulsion during neural development (15). Furthermore, 14-3-3s regulate growth cone turning responses through protein kinase A (16). Both processes involve altering the morphology of growth cones through cytoskeletal rearrangements (17). Therefore, 14-3-3s may mediate pathways that modulate cytoskeletal rearrangements. The observed growth of MBP-positive oligodendrocytes and OPCs due to 14-3-3 activity inhibition may also be regulated by such pathways. Moreover, 14-3-3 ζ inhibition impairs actomyosin contraction in human trabecular meshwork cells of the

eye through the RhoA signaling pathway (18). This further demonstrates the implication of 14-3-3s in regulating cytoskeletal structures.

This study showed that inhibition of 14-3-3 protein activity results in increased growth of MBP-positive cells. This is consistent with previous results that demonstrated reduced differentiation of OPCs to oligodendrocytes due to the administration of the 14-3-3-client protein interaction stabilizer, fusicoccin. These findings suggest that inhibition and upregulation of 14-3-3 protein activity may increase and decrease the growth of maturing OPCs, respectively. This research expands the current understanding of the role of 14-3-3 proteins in oligodendrocyte maturation and may pioneer therapeutic strategies to ameliorate demyelination diseases such as multiple sclerosis (19).

Figures



(A) Primary OPCs 2 days in vitro treated with either 1μM BV02 or control (CT) DMSO. **(B)** Area covered normalized to the control group by MBP-positive cells in experimental groups treated with either BV02 or control DMSO. **(C)** Area covered normalized to the control group by MBP-positive cells in experimental groups transduced with either difopein or control WLRL. **(D)** Primary OPCs 2 days in vitro transfected with either difopein or control WLRL plasmid. **(E)** Percentage of cleaved caspase 3-positive cells for difopein experiment for both MBP-positive and MBP-negative cells. **(F)** Expression of different 14-3-3 isoforms in OPCs detected by western blot 2 and 5 days in vitro. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene. * $p < 0.05$ ** $p < 0.01$ **** $p < 0.0001$.

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