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Research Report

Altered glycosylation of α-dystroglycan in neurons of Fukuyama congenital muscular dystrophy brains

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FCMD, Fukuyama congenital muscular dystrophy
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ABSTRACT

To test the hypothesis that the disruption of fukutin protein produces the brain pathology through hypoglycosylation of α-dystroglycan (α-DG), we immunostained Fukuyama congenital muscular dystrophy (FCMD) brains with an antibody that recognizes the polysaccharide epitope of α-DG. Immunoreactivity of the glia-limitans along the cortical surface, as well as that of the glial endfeet around vessel walls, was preserved in the FCMD cerebrum. However, fragmentation of the immunostained glia-limitans was noted in association with parenchymal protrusion and gyral fusion. In the FCMD cerebellum, this fragmentation of α-DG labeling was limited to the area of micropolygyria, and immunostaining at the glia-limitans and vessel walls was comparable to that of the control brains, in structurally normal areas. In the hippocampus, neurons of the dentate gyrus and corpus ammonis were immunopositive for α-DG in control subjects, but this staining was markedly decreased in FCMD brains. In contrast, immunolabeling of blood vessels and the glia-limitans was preserved in this region. Fukutin antisera clearly labeled hippocampal neurons in control brains, while this labeling was decreased in FCMD brains. Thus, hypoglycosylation of α-DG was evident in neurons, but not in the glial cell population of FCMD brains. This suggests that the mechanism of α-DG glycosylation may differ between neurons and glial cells, and that a fukutin gene defect may result in functional disruption through hypoglycosylation of both neuronal and glial α-DG.

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1. Introduction

Fukuyama congenital muscular dystrophy (FCMD) is caused by mutations in the fukutin gene (Kobayashi et al., 1998), the product of which is involved in the glycosylation of α-dystroglycan (α-DG) (Aravind and Koonin, 1999; Michele et al., 2002), a central component of the dystrophin–glycoprotein complex (Ervasti and Campbell, 1993; Henry and Campbell, 1999; Ibraghimov-Beskrovnaya et al., 1992). Hypoglycosylation of α-DG results in the decreased binding of laminin at the sarcolemma and in the subsequent dystrophic pathology of skeletal muscles (Michele et al., 2002).

As for the central nervous system, FCMD brains are characterized by cerebral and cerebellar micropolygyria (Kamoshita et al., 1976). Based on the breached glia-limitans and the protrusion of glial–neuronal tissue into the subarachnoid space, a fragile glia-limitans has been hypothesized to be a cardinal feature of the pathological process in FCMD brains (Nakano et al., 1996; Takada et al., 1987). The presence of α-DG in the glia-limitans, and in the glial endfeet of vessel walls (Michele et al., 2002; Zaccaria et al., 2001), supports the hypothesis that hypoglycosylation of α-DG results in decreased binding of glial α-DG with extracellular matrix proteins. Decreased integrity of the glia-limitans may be responsible for the protrusion of brain parenchyma and overmigration of neurons beyond the pial surface during cerebral corticogenesis. However, in contrast to the findings in muscle tissue, hypoglycosylation of α-DG has not been identified in the glia-limitans of FCMD brains. In addition, fukutin protein has been localized in both neurons and glial cells (Ohtsuka-Tsurumi et al., 2004; Saito et al., 2000; Sasaki et al., 2000; Yamamoto et al., 2002). In this study, we immunostained FCMD brains with an antibody that recognizes the polysaccharide epitope of α-DG. Interestingly, the immunoreactivity of glycosylated α-DG in the subpial glia-limitans was not ubiquitously disrupted. On the other hand, hypoglycosylation of α-DG was suggested in hippocampal neurons of FCMD brains. This differential hypoglycosylation may provide a clue to understanding the pathological process in FCMD brains.

2. Results

The α-DG antibody labeled the glia-limitans at the cortical surface and the vessel walls in the control cerebrum (Fig. 1A) and cerebellum (Fig. 1D). In FCMD brains, the continuity of glia-limitans staining was occasionally interrupted at regions with parenchymal protrusions beyond the pial surface (Fig. 1B). Fragmentation of α-DG immunopositive membrane was also noted at the gyral fusion (Fig. 1C). In the FCMD cerebellum, immunostaining at the glia-limitans and vessel walls was comparable to that in the control brains, in areas without micropolygyria (Fig. 1E). The fragmentation of α-DG labeling at the glia-limitans was restricted to the area of micropolygyria (Fig. 1F). Laminin immunolabeling was fragmented and correlated with that of α-DG in the structurally disordered regions of FCMD cerebrum and cerebellum (Figs. 2A and B). α-DG immunolabeling on capillary walls was confirmed by double staining with antifactor VIII (Figs. 2C to E). In the hippocampus, neurons of the dentate gyrus and the corpus ammonis were immunopositive for α-DG in control subjects (Figs. 3A and C), but this staining was markedly decreased in all of the FCMD brains (Figs. 3B and D). In contrast, immunolabeling of blood vessels and the glia-limitans was preserved in this region. The immunolabeling disappeared completely in negative control experiments (not shown). Other than the hippocampus, α-DG immunolabeling was not detected in the cerebral and cerebellar neurons of either control or FCMD brains.

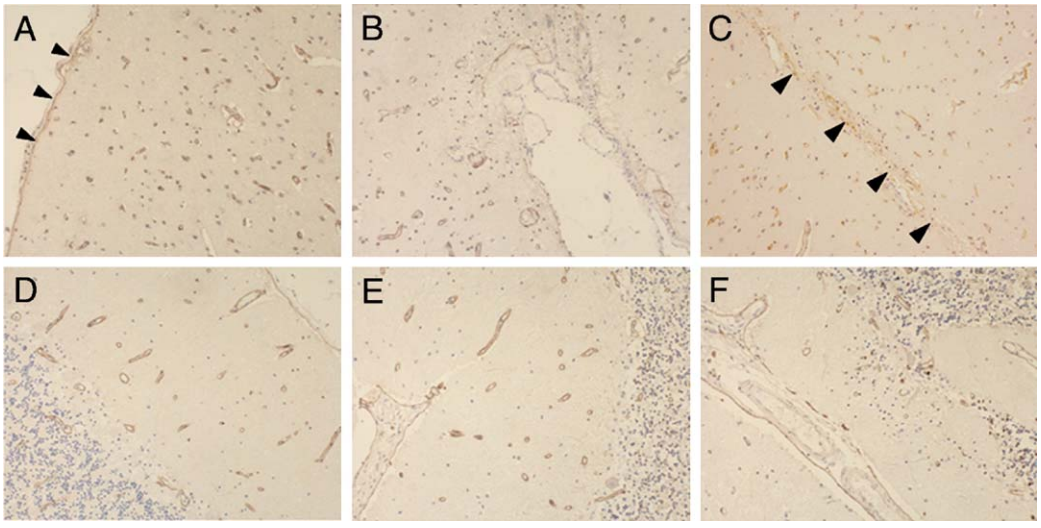

Fig. 1 – α-dystroglycan (α-DG) immunoreactivity in control (A and D) and FCMD (B, C, E, and F) brains. The α-DG positive glia-limitans is continuous in the control cerebrum (arrowheads in A), but fragmentary on the cortical surface (B) and at the cortical fusion (C, arrowheads) in FCMD brains. The continuity of the α-DG positive glia-limitans is preserved in the control cerebellum (D) and in the FCMD cerebellum in areas with normal structure (E), but is disrupted in the FCMD cerebellum in areas of micropolygyria (F). A to F: 200×.