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Chapter

Multiple Functions of *Fukutin*, the Gene Responsible for Fukuyama Congenital Muscular Dystrophy, Especially in the Central Nervous System

Tomoko Yamamoto, Yukinori Okamura, Ryota Tsukui, Yoichiro Kato, Hiromi Onizuka and Kenta Masui

Abstract

Fukuyama congenital muscular dystrophy (FCMD), accompanying central nervous system (CNS) and ocular anomalies, is the second common muscular dystrophy in Japan, and the responsible gene is *fukutin*. The lesions are mainly caused by fragile basement membrane/cell membrane due to hypoglycosylation of α-dystroglycan (α-DG), and astrocytes play a crucial role for CNS malformation. On the other hand, since fukutin is expressed almost ubiquitously, diverse functions of fukutin, besides the glycosylation of α-DG, can be considered. As for the CNS, fukutin possibly upregulates cyclin D1 expression as a cofactor of activator protein-1 in astrocytoma. Moreover, fukutin may be involved in the phosphorylation of tau, one of the key proteins of dementia represented by Alzheimer’s disease, in glutamatergic neurons. A presynaptic function in GABAergic neurons is also suggested. Owing to the recent advances of molecular and biochemical techniques, new therapeutic strategies are under consideration, even for brain malformation, which begins to be formed during the first trimester *in utero*. Recovery of hypoglycosylation of α-DG supposed to be a main therapeutic target, but to know various functions and regulation systems of fukutin might be important for developing suitable therapies.

Keywords: fukutin, multifunction, cell proliferation, astrocyte, tau phosphorylation, glutamatergic neuron, presynaptic function, GABAergic neuron

1. Introduction

Fukuyama congenital muscular dystrophy (FCMD), an autosomal recessive disease firstly reported by Fukuyama et al. in 1960 [1], is the second common muscular dystrophy in Japan [2]. It is one of the muscular dystrophies accompanies central...
nervous system (CNS) and ocular anomalies and is included in α-dystroglycanopathy [3, 4]. The responsible gene is *fukutin* [5]. α-dystroglycan (α-DG), a glycoprotein and a component of dystrophin-glycoprotein complex at the cell/basement membrane, binds to some basement membrane proteins, such as laminin-α2, neurexin-α, and agrin [4, 6]. The sugar chain works as a receptor [4, 6]. The expression is not only in the skeletal muscle but also in the CNS [7] and other organs [8]. Hypoglycosylation of α-DG causes fragile basement membrane, which is considered for the major pathogenesis of α-dystroglycanopathy. Several proteins including fukutin are involved in the glycosylation of α-DG [4, 6]. Fukutin has a function of ribitol 5-phosphate (Rbo5P) transferase that transfers Rbo5P from cytidine diphosphate-Rbo to α-DG [9]. α-DG is hypoglycosylated in the skeletal and cardiac muscles of FCMD patients [10]. Like α-DG, fukutin is expressed in various organs, almost ubiquitously [5, 8]. Diverse functions of fukutin can be suggested. To know these functions seems helpful not only for better understanding of FCMD pathology, but also for developing new therapeutic strategies. In this chapter, several intriguing roles of fukutin in the CNS are presented.

2. Fukutin gene

*fukutin* is localized on chromosome 9q31 and encodes a 461-amino-acid protein [5]. The mRNA of 7349 bp contains an open reading frame of 1383 bp, composed of 10 exons, and a long 3′-untranslated region [5]. Alternative splicing has been found [11]. In FCMD, a common genetic mutation is a retro-transposal insertion of about 3000 bp into the 3′-untranslated region, called founder haplotype [5], but other mutations have been reported [11]. Mutations heavily affecting the coding protein may provoke a severe phenotype resembling Walker-Warburg syndrome [12–14], while those influencing lightly may cause mild phenotypes like limb girdle muscular dystrophy [12, 15, 16].

3. Clinicopathological characteristics of FCMD

Generally, FCMD patients are born as floppy infants, and peak motor function is achieved around 5 years old [2]. Patients usually die before 20 years old, but milder cases may live around 30 years old. Besides muscular dystrophy of the skeletal muscle, cardiac involvement is known. As clinical manifestations of the CNS lesion, mental retardation is observed, and more than 50% of patients show seizures. Ophthalmologic symptoms, such as myopia and abnormal eye movements, can be seen.

The CNS lesion of FCMD is represented by cobblestone lissencephaly, in other words polymicrogyria, of the cerebrum and cerebellum in post-natal cases [17, 18] (Figure 1). Abnormalities in the spinal cord may be found, especially in severe cases [19]. In the cerebrum and cerebellum of fetal cases, the glia limitans, covered with the basement membrane of the brain surface, is disrupted [20, 21]. In the cerebrum, overmigration of glioneuronal tissues through disruptions is obvious (Figure 1). The basement membrane/cell membrane at the glia limitans is abnormal, electron microscopically [21, 22]. Immunoreaction against anti-glycosylated α-DG [19] and laminin-α2 [23] antibodies is decreased at the glia limitans.
4. Functions of Fukutin in astrocytes

4.1 Functions related to the glycosylation of α-DG

The major pathogenesis of the polymicrogyria of FCMD is considered to be a fragile basement membrane due to hypoglycosylation of α-DG, which causes the disruption of the glia limitans [19]. Disruptions are already detectable in a fetus of 16 weeks of the gestation (Figure 1). Since astrocytes, expressing both α-DG [24] and fukutin [25], form the glia limitans, astrocytes are mainly involved in the pathogenesis of CNS lesions of FCMD [19]. The cerebrum and cerebellum show different histological appearances in polymicrogyria, owing to the difference of their structures, components, and ways of neuronal migration. In post-natal FCMD cases, the cerebral surface is continuous, exhibiting marked superficial gliosis with obvious elongation of astrocytic endfeet [19]. After maturation, astrocytes may be able to compensate the fragile basement membrane/cell membrane by reactive gliosis.

4.2 Functions other than the glycosylation of α-DG: relation to cell proliferation

In astrocytes, involvement in the glycosylation of α-DG is the most important role for the pathogenesis of CNS lesions of FCMD. However, other function of fukutin has been found, regarding the regulation of cell proliferation. On an astrocytoma cell line (1321 N1) highly expressing cyclin D1, cell proliferation and expression of cyclin D1 are decreased by suppression of fukutin and increased by overexpression (Figure 2) [26]. Cyclin D1, one of the proteins controlling the cell cycle, facilitates cells entering into the S phase of cell cycle for cell proliferation, and its expression is regulated by various transcription factors [27]. In the promoter area of cyclin D1, there are multiple binding sites for each transcription factor, including activator protein-1 (AP-1) [27]. It has been found that a complex
containing fukutin protein binds to the AP-1 binding site of cyclin D1 and fukutin protein and AP-1 are co-localized [26]. Fukutin can take part in the transcription regulation of cyclin D1 as a cofactor of AP-1, independent from the glycosylation of α-DG [26].

Astrocytes play a variety of roles to maintain the function of CNS properly, among which tissue repair is included. Activated astrocytes proliferate and migrate to repair damaged areas [28]. Given that fukutin is involved in the cell proliferation, a loss of fukutin in astrocytes might influence to wound healing in the CNS of FCMD patients, although studies have not been performed from this point of view to the author’s knowledge. Apart from the pathogenesis of FCMD, it might be intriguing to investigate about fukutin on the standpoint of cell proliferation of astrocytoma. Fukutin might act as a cofactor of some other transcription factors besides AP-1.

5. Functions of fukutin in neurons

5.1 Functions in immature neurons

Although astrocytes are considered to play a crucial role to form the CNS lesions of FCMD, fukutin is also expressed in immature and mature neurons [25, 29]. In the cerebrum, immature neurons migrate from the ventricular zone to the cortical plate along with cytoplasmic processes of radial glia during the early fetal period [30, 31]. The migration is almost completed by 20–24 weeks of gestation [31]. Extracellular matrix proteins such as laminin and agrin are indispensable for the attachment of immature neurons and cytoplasmic processes of radial glia [32]. Since the sugar chain of α-DG is a receptor of such extracellular matrix proteins [4, 6], the glycosylated α-DG seems necessary for the neuronal migration [33]. Neurons begin to differentiate after settling to the proper place of the cerebral cortex [31]. Fukutin expression in immature neurons seems reasonable for the neuronal migration, smoothly interacting with radial glial fibers while keeping immaturity [34]. Irregular distribution of immature neurons is observed in the severely affected area of the cerebrum of FCMD, indicating that migration arrest may be apparent when a function of fukutin is seriously damaged [19]. Compensation of fukutin function by other proteins may be more established in neurons than in astrocytes.
5.2 Functions of fukutin in mature neurons

5.2.1 Function in glutamatergic neurons, with relation to the phosphorylation of tau

Fukutin expression is decreased in mature neurons [34], but some functions must exist in mature neurons. In the brain of FCMD patients more than 20 years old, neurofibrillary tangles (NFTs) immunopositive for phosphorylated tau (p-tau) are predominantly observed in the cerebral cortex [35, 36]. In our adult case of FCMD, NFTs are exclusively observed in areas showing polymicrogyria, and not found in the occipital robe showing almost normal appearance. In the CNS, there are excitatory and inhibitory neurons. The majority of neurons that constitute the cerebral cortex are excitatory/glutamatergic neurons, using glutamate as neurotransmitter, and the rests are inhibitory neurons [30]. Among several types of inhibitory neurons, neurons using γ-aminobutyric acid (GABA) as a neurotransmitter, GABAergic neurons, are a main component in the cerebral cortex [30]. GABA is synthesized from glutamate by glutamate decarboxylase (GAD) [37], which is used as a marker of GABAergic neurons. On immunohistochemical examination of the cerebrum of FCMD cases, neurons containing p-tau-positive NFT do not express GAD. NFTs are likely to be formed in excitatory neurons (Figure 3) [38]. Abnormal architectures of neurons derived from the disruption of glia limitans may be one of the factors to give rise to NFTs, considering from the distribution of neurons containing NFT. On the other hand, it is imaginable that fukutin itself is involved in the formation of NFTs.

In the adult CNS, there are six tau isoforms, which are divided into three microtubule-binding repeat (3R) and four microtubule-binding repeat (4R), depending on the absence or existence of exon 10 [39, 40]. There are various neurodegenerative diseases exhibiting p-tau-positive inclusions, so-called tauopathy. Three types of p-tau accumulation are known: 3R + 4R tauopathy, in which inclusions contain 3R and 4R tau, is represented by Alzheimer’s disease; 3R tauopathy represented by Pick’s disease; 4R tauopathy represented by corticobasal degeneration [39]. On FCMD, Western blotting [36] and immunohistochemical examination reveal both 4R and 3R tau in the diseased brain (Figure 3). There is something common on formation of p-tau-positive NFTs in FCMD and Alzheimer’s disease. However, the distribution of NFTs is somewhat different. Alzheimer’s disease shows NFTs predominantly distributed in

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Figure 3.
Immunohistochemistry on the cerebrum of a 27-year-old FCMD patient. On double-immunohistochemical staining, p-tau (brown)-positive NFT is not formed in a glutamate decarboxylase (purple)-positive neuron (A), NFT is positive for both 3R tau (B) and 4R tau (C). NFT: Neurofibrillary tangle, GAD: Glutamate decarboxylase, 3R: Three microtubule-binding repeats, 4R: Four microtubule-binding repeats.
the limbic system and in the cerebral cortex, accompanying with senile plaques [41]. In contrast, NFTs are tended to be more in the cerebral cortex, and senile plaques are not found in FCMD. Different pathogenesis can be assumed.

Many molecules are involved in the phosphorylation of tau. One of the representative proteins is glycogen synthase kinase-3 (GSK-3β) [42]. Using neuroblastoma cell lines, we have found that the phosphorylation of both tau and GSK-3β is augmented by suppression of fukutin and is reduced by overexpression of fukutin [38]. Moreover, fukutin, tau, and GSK-3β are suggested to form a complex (Figure 4) [38]. Fukutin is possibly involved in the phosphorylation of tau, mediated by GSK-3β, which appears to be independent from the glycosylation of α-DG. It is likely that on glutamatergic neurons of the FCMD cerebrum, loss of fukutin accelerates the phosphorylation of tau, which may be augmented by abnormal network of neurons. Implication of GSK-3β is considered for this phosphorylation. However, GSK-3β is involved in the

![Image](image.png)

**Figure 4.**
Double-immunocytochemical staining on neuroblastoma cells (SH-SY5Y). Fukutin and tau are co-localized (A-C). Fukutin and glycogen synthase kinase-3 show similar localization (D-F). No immunoreaction is observed in negative controls (G-I). FKTN: Fukutin, GSK-3β: Glycogen synthase kinase-3 β.
abnormal accumulation of amyloid-\(\beta\) as well, a main component of senile plaque [42, 43]. Further studies are required to elucidate the mechanism between fukutin and tau phosphorylation.

5.2.2 Function in GABAergic neurons, with relation to the synaptic function

In the cerebrum of our adult FCMD patient, immunoreaction against anti-GAD antibody is increased [38]. Several factors can be postulated for the explanation, e.g., compensation toward hyperactivities of glutamatergic neurons, reaction against abnormal postsynaptic or presynaptic functions, etc. It is curious to know whether fukutin itself directly implicated in this phenomenon or not. A loss of fukutin appears to trigger the increase of GAD, because the increase is observed throughout the cerebral cortex, including the occipital robe showing almost normal histological appearance [38]. The dystrophin-dystroglycan complex (DGC) is existed in the postsynapse, and postsynaptic function of \(\alpha\)-DG is well known [6, 44]. In contrast, studies about presynaptic functions of the DGC are not so many, but the DGC exists in the presynapse of GABAergic neurons [45]. On neuroblastoma cell lines, fluorescence immunocytochemistry has revealed that expression GAD is increased by suppression of fukutin and decreased by overexpression of fukutin [38]. Co-localization of fukutin, GAD, and synaptophysin is also suggested (Figure 5) [38]. Since synaptophysin is a component of presynaptic vesicle [46], co-localization of fukutin and synaptophysin supports presynaptic function of fukutin. From the existence of the DGC at the presynapse, increase of GAD in GABAergic neurons might result from the decreased glycosylation of \(\alpha\)-DG, but co-localization of fukutin and GAD might indicate a direct involvement of fukutin.

![Figure 5](image)
6. Future perspectives

In addition to the glycosylation of $\alpha$-DG, fukutin can contribute at least to cell proliferation, tau phosphorylation, and presynaptic function. The Golgi apparatus is considered to be a major subcellular localization [5, 47], because fukutin is involved in the glycosylation of $\alpha$-DG. However, PSORT II prediction favors localizations of fukutin in the cytoplasm, mitochondria and nucleus rather than the Golgi apparatus. This prediction matches the findings presented in this chapter and suggests more unknown functions of fukutin.

With regard to the phosphorylation of tau, a relation between fukutin and microtubules can be assumed. Tau is a microtubule-binding protein to stabilize microtubules. Phosphorylated tau proteins that cannot bind to the microtubules are accumulated in the cytoplasm, resulting in NFTs [40]. Fukutin could be involved in the stabilization of microtubules by suppressing the phosphorylation of tau. When fukutin is knocked down on neuroblastoma cell lines, cytoplasmic processes are elongated (Figure 6) [34]. Elongation of cytoplasmic processes is also observed in fukutin-suppressed astrocytoma cells [48]. Astrocytes express tau, and astrocytic tau pathology has been reported [49]. It has been shown that overexpression of tau disturbs movements of kinesin, one of the representative molecular motors, and tau-stable cells exhibit rather round appearances [50]. Fukutin might relate to functions of microtubules via tau phosphorylation, not only influencing their stabilization but also affecting movements of molecular motors and cell morphology. A relation between fukutin and microtubules is proposed in cardiomyocytes as well [51]. To study more about the relation between fukutin and microtubules appears interesting.

To mention more about glial cells, there are four major types of glial cells in the CNS; astrocyte, oligodendrocyte, microglia, and ependymal cell. Functions of fukutin in astrocytes are indispensable for the pathogenesis of the CNS lesion of FCMD, while functions in other glial cells have not been elucidated. There are only a few observations suggesting functions of fukutin in oligodendrocytes. The cerebral white matter of FCMD exhibits dysmyelination [52], and fukutin-deficient chimera mice show loss of myelination in the peripheral nerve [53]. On microglia and ependymal cells, investigations relating to fukutin have not been found in English literatures to the authors’ knowledge.

![Figure 6](image)

**Figure 6.** Morphological alteration of neuroblastoma cells (IMR32) after suppression of fukutin. Elongation of cytoplasmic processes is conspicuous on cells with suppression of fukutin (A), compared with control cells (B). KD: Knockdown of fukutin.
As for therapies of FCMD, in addition to conventional treatments, efficacy of steroids [54] and rapamycin [55] has been suggested for muscular dystrophy. A retro-transposal insertion in the 3′-untranslated region of fukutin provokes pathogenic exon-trapping, resulting in a production of abnormal fukutin protein [56]. Treatment of antisense oligonucleotide can prevent this pathogenic exon trapping and restore normal fukutin production on human primary myotube obtained from FCMD patients and from FCMD model mice knocked in the retro-transposal insertion [56]. CDP-ribitol prodrug ameliorates muscular dystrophy in mice that lack isoprenoid synthase domain-containing protein (ISPD), one of the causative genes of α-dystroglycanopathy [57]. Surprisingly, recent studies propose novel strategies toward brain malformation, despite the CNS and ocular anomalies begin to be formed during the first trimester in utero. Severe brain malformation of Emx1-fukutin-cKO mouse is prevented by delivery of fukutin into the brain at E12.5 [58]. In a brain organoid model of FCMD, abnormal radial glial fiber migration is restored by Mannan-007 [59]. It is not easy to overcome a lot of difficulties to apply new molecular or gene-based therapies, especially to fetuses. Unexpected phenomena could happen. On developing new therapeutic strategies, especially of molecular level, a good knowledge about functions and regulation systems of fukutin seems necessary.

7. Conclusion

Fukutin is considered to be multifunctional. In the CNS, fukutin can be involved at least in the cell proliferation, tau phosphorylation, and presynaptic function, some of which seems independent from the glycosylation of α-DG. To see fukutin form various standpoints may be interesting and indispensable, not only for deep understanding of FCMD pathology but also for developing suitable therapies.

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