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TFEB activation promotes the recruitment of lysosomal glycohydrolases β-hexosaminidase and β-galactosidase to the plasma membrane

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ABSTRACT

Lysosomes are membrane-enclosed organelles containing acid hydrolases. They mediate a variety of physiological processes, such as cellular clearance, lipid homeostasis, energy metabolism and pathogen defence. Lysosomes can secrete their content through a process called lysosome exocytosis in which lysosomes fuse with the plasma membrane realising their content into the extracellular milieu. Lysosomal exocytosis is not only responsible for the secretion of lysosomal enzymes, but it also has a crucial role in the plasma membrane repair. Recently, it has been demonstrated that lysosome response to the physiologic signals is regulated by the transcription factor EB (TFEB). In particular, lysosomal secretion is transcriptionally regulated by TFEB which induces both the docking and fusion of lysosomes with the plasma membrane. In this work we demonstrated that TFEB nuclear translocation is accompanied by an increase of mature glycohydrolases β-hexosaminidase and β-galactosidase on cell surface. This evidence contributes to elucidate an unknown TFEB biological function leading the lysosomal glycohydrolases on plasma membrane.

1.Introduction

Lysosomes are involved in the degradation of a wide variety of structurally diverse substances into their basic building blocks, such as proteins, glycosaminoglycans, glycogen, nucleic acids, oligosaccharides and sphingolipids [1]. The coordinated expression of lysosomal genes is essential not only for the degradation and recycling of metabolic intermediates but also for other specific cellular processes in which lysosomes are involved, such as cell-surface receptor regulation and antigen presentation [2–4].

Mutations that cause lysosomal enzymes deficiency are at the basis of a group of more than 50 genetic disorders, called Lysosomal Storage Disorders (LSDs), characterised by the accumulation of autophagic and endosomal substrates. Most of LSDs are associated with abnormal brain development and mental retardation. In addition, they are characterised by intracellular deposition and protein aggregation, events also found in age-related neurodegenerative disorders, such as Alzheimer's and Parkinson's Diseases [5–8]. The clinical phenotypes observed in LSDs are not a simple consequence of the accumulation of a specific substrate in lysosomes, but they are rather associated with an impairment of endosomal–lysosomal trafficking and autophagy [9,10].

Currently, many reports indicate the association of glycohydrolases with the plasma membrane [11]. In particular, neuraminidase 3,β-hexosaminidase, β-galactosidase and β-glucosidase, working on the oligosaccharide chain of glycosphingolipids, are associated with the external leaflet of the plasma membrane, where they display both *cis* and *trans* activity, being capable to act as well on surface components of the neighbouring cells [12–16]. It has been also demonstrated that recruitment on plasma membrane of functionally related glycohydrolases increases in cells overexpressing the α-subunit of β-hexosaminidase [17]. Moreover, the association of fully processed β-hexosaminidase and β-galactosidase with plasma membrane lipid microdomains has been demonstrated [18].

β-hexosaminidase (Hex, EC 3.2.1.52) and β-galactosidase (Gal, EC 3.2.1.23) are both involved in the stepwise degradation of GM1 to GM3 ganglioside. Hex is an acidic glycohydrolase that cleaves terminal β-linked N-acetylglucosamine or N-acetylgalactosamine residues from oligosaccharides, glycolipids, glycoproteins and glycosaminoglycans [19], while Gal catalyses the

Abbreviations: flot-2, flotillin-2; Gal, β-galactosidase; GM1, Galβ1,3GalNAcβ1,4-(NeuAcα2,3)-Galβ1,4Glc-ceramide; GM2, 3GalNAcβ1,4-(NeuAcα2,3)-Galβ1,4Glc-ceramide; GM3, NeuAcα2,3Galβ1,4Glc-ceramide; GSLs, glycosphingolipids; Hex, β-hexosaminidase; MUGal, 4-methylumbelliferyl-β-D-galactopyranoside; MUG, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide; MUGS, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide-6-sulphate; TFEB, transcription factor EB.

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hydrolysis of terminal N-linked galactosyl moiety from oligosaccharides and glycosides [20]. Genetic deficiency of Hex and Gal leads to the occurrence of GM2 and GM1 gangliosidosis, respectively, two severe neurodegenerative LSDs [19]. Moreover, alterations of these enzymes and their membrane-associated forms have been observed in pregnancy [21], apoptosis [22], neurodegenerative disorders [8,23], and cancer [24].

Recently, it has been demonstrated that the adaptive response of lysosomes to physiologic changes is related to the activity of TFEB [25]. TFEB is a master gene that induces autophagy, lysosome biogenesis and up-regulation of lysosomal genes expression [26,27]. Moreover, it has been reported that TFEB induces the clearance of storage material in several LSD cell models by promoting lysosomal exocytosis [28], which is in turn responsible for the secretion of lysosomal content in extracellular environment and plasma membrane repair [29].

In this work, we overexpressed TFEB in HEK-293 cells and analysed cell surface-associated Hex and Gal enzymes to explore whether TFEB also modulates the levels of plasma membrane-associated glycohydrolases.

2. Materials and methods

2.1. Cell culturing

HEK-293 cells (ATCC, Manassas, VA, USA), were cultured in DMEM supplemented with 10% (v/v) heat-inactivated foetal bovine serum, 2 mM L-glutamine, 100 units/ml penicillin, 100 mg/ml Streptomycin in a humidified incubator under 5% CO_2 at 37 °C. Starvation was performed in HBSS medium (Sigma–Aldrich), with Ca and Mg, supplemented with 10 mM HEPES. The viability of the cells was estimated by examining their ability to exclude trypan blue.

2.2. TFEB cloning and cells transfection

Total RNA was extracted from HEK-293 cells with PureLink™ Total RNA Purification System (Invitrogen). cDNA was obtained by reverse transcription of total RNA with random hexamer primers and SuperScript™ II Reverse Transcriptase according to the manufacturer's procedure (Invitrogen). Finally, full-length human TFEB cDNA was obtained by PCR performed with Phusion® Hot Start II (Finzymes) using the following primers: i) forward 5′-ATGGTACCC CACCATGGCGTCACGCAT-3′; reverse 5′-ATGAATTC-TCACAGCACATCGCCCTCC-3′; ii) forward 5′-ATGAATTCCCACCATG-GCGTCACGCA-3′; reverse 5′-ATGGTACCGTCAGCA CATCGCCCTCC TC-3′. Full-length human TFEB was cloned (i) into the pcDNA™6/*myc*-His A vector (Invitrogen) to produce TFEB without tag; (ii) into the pEGFP-N1 vector in frame with EGFP cDNA. TFEB–EGFP cDNA was successively subcloned in pcDNA™6/*myc*-His A vector to obtain TFEB–EGFP. Cells were seeded in six-well plates at 70% confluence before transfection. Transfection was performed by using jetPEI™ (Polyplus transfection) according to the manufacturer's protocols. Transfectants for TFEB and TFEB–EGFP (TFEB cells and TFEB–EGFP cells) and with the empty vector (CTRL cells) were selected with 8 μg/ml Blasticidin (Sigma–Aldrich).

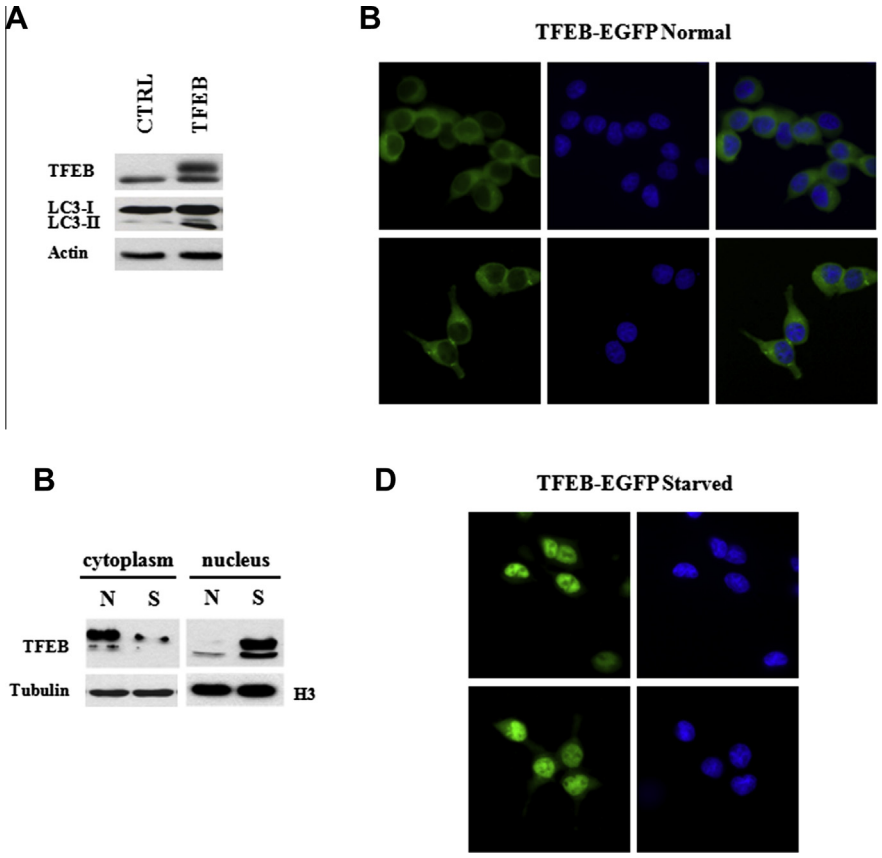

Fig. 1. Cells starvation promotes TFEB nuclear translocation. (A) Immunoblot analysis showing TFEB, LC3 and actin levels in extracts (30 μg of protein) from CTRL and TFEB cells. Representative immunoblotting of three independent experiments is reported. (B) Immunoblot analysis showing TFEB levels in cytosolic (30 μg of proteins) and nuclear (5 μg of proteins) extracts from TFEB cells, both in normal (N) and starved (S) conditions. Tubulin and H3 were used as cytosolic and nuclear markers, respectively. Representative immunoblotting of three independent experiments is reported. (C, D) Fluorescent microscopy images of TFEB–EGFP cells, both in normal and starved conditions. Magnification, 60×.