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Research Paper

Murine Sialidase Neu3 facilitates GM2 degradation and bypass in mouse model of Tay-Sachs disease

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ABSTRACT

Tay-Sachs disease is a severe lysosomal storage disorder caused by mutations in *Hexa*, the gene that encodes for the α subunit of lysosomal β-hexosaminidase A (HEXA), which converts GM2 to GM3 ganglioside. Unexpectedly, *Hexa*$^{-/-}$ mice have a normal lifespan and show no obvious neurological impairment until at least one year of age. These mice catabolize stored GM2 ganglioside using sialidase(s) to remove sialic acid and form the glycolipid GA2, which is further processed by β-hexosaminidase B. Therefore, the presence of the sialidase (s) allows the consequences of the *Hexa* defect to be bypassed. To determine if the sialidase NEU3 contributes to GM2 ganglioside degradation, we generated a mouse model with combined deficiencies of HEXA and NEU3. The *Hexa*$^{-/-}$*Neu3*$^{-/-}$ mice were healthy at birth, but died at 1.5 to 4.5 months of age. Thin-layer chromatography and mass spectrometric analysis of the brains of *Hexa*$^{-/-}$*Neu3*$^{-/-}$ mice revealed the abnormal accumulation of GM2 ganglioside. Histological and immunohistochemical analysis demonstrated cytoplasmic vacuolation in the neurons. Electron microscopic examination of the brain, kidneys and testes revealed pleomorphic inclusions of many small vesicles and complex lamellar structures. The *Hexa*$^{-/-}$*Neu3*$^{-/-}$ mice exhibited progressive neurodegeneration with neuronal loss, Purkinje cell depletion, and astrogliosis. Slow movement, ataxia, and tremors were the prominent neurological abnormalities observed in these mice. Furthermore, radiographs revealed abnormalities in the skeletal bones of the *Hexa*$^{-/-}$*Neu3*$^{-/-}$ mice. Thus, the *Hexa*$^{-/-}$*Neu3*$^{-/-}$ mice mimic the neuropathological and clinical abnormalities of the classical early-onset Tay-Sachs patients, and provide a suitable model for the future pre-clinical testing of potential treatments for this condition.

1. Introduction

The GM2 gangliosidoses (Tay-Sachs, OMIM 272800; Sandhoff, OMIM 268800; and GM2AP deficiency, OMIM 272750) are a group of rare lysosomal storage disorders caused by mutations in three different genes: *Hexa*, *Hexb*, and *Gm2ap*. *The Hexa* and *Hexb* genes encode the α and β subunits, respectively, of β-*N*-acetylhexosaminidase, (HEXA, αβ, EC 3.2.1.52), which form a complex with GM2AP to hydrolyze GM2 ganglioside and remove *N*-acetylgalactosamine (Gravel et al., 2001). GM2 ganglioside is a glycosphingolipid that serves as an intermediate in complex brain ganglioside (mainly GM1a, GD1a, GD1b, and GT1b) biosynthesis and degradation (Kolter and Sandhoff, 1999). Complex brain gangliosides are most abundant in the cell membranes of neurons, where they contribute to axon-myelin interactions, Ca^{2+} homeostasis, and the activity of a diverse range of trans-membrane receptor signaling pathways (Regina and Hakomori, 2008; Schnaar, 2010; Ohmi et al.,

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2012; Nordstrom et al., 2013; Allende and Proia, 2014). Therefore, the central nervous system is the most impacted organ in patients because of the high levels of the HEXA substrate, GM2 ganglioside. Tay-Sachs disease is clinically similar to Sandhoff disease and GM2-activator deficiency (Sandhoff, 2016). The classic infantile acute form begins early in infancy and leads to death by four years of age. It is also known as early-onset type I GM2 gangliosidosis (B variant or pseudo-AB variant). Variable phenotypes with a range of disease severities and ages of onset result when low levels of residual HEXA activities are present (Sandhoff and Conzelmann, 1984). Juvenile subacute (the onset can be between two and ten years of age) and chronic (onset in adult life) forms of the disorder can occur. Patients with the rapidly progressive and acute form of this disorder exhibit a total or near-total loss of HEXA activity that is mostly due to missense or nonsense mutations. Diagnosis can be made by a number of methods, such as measurement of HEXA enzyme activity using synthetic substrates. The clinical symptoms of progressive neurodegeneration and developmental delay are caused by the accumulation of GM2 ganglioside in lysosomes, which leads to early cell death in Tay-Sachs patients.

Two independent studies attempting to generate mouse models of GM2 gangliosidoses by targeting the mouse *Hexa* and *Hexb* genes yielded unexpected results (Phaneuf et al., 1996; Yamanaka et al., 1994). The disruption of *Hexb* resulted in a severe neurological phenotype similar to Sandhoff disease; however, the disruption of *Hexa* resulted in nearly normal mice rather than a severe early-onset neurological phenotype. These findings were explained by the presence of one or more sialidases in mice that can remove sialic acid from GM2 ganglioside to create GA2 ganglioside, which can then be degraded by the HEX B (ββ) present in HEXA- deficient Tay-Sachs mice (Fig. 1A) (Sango et al., 1995; Yuziuk et al., 1998). The generation of a HEXB-deficient Sandhoff disease model in mice was useful for the initial evaluation of potential therapies for the GM2 gangliosidoses. However, $Hexa^{-/-}$ mice have proven to be of little value as a model for Tay-Sachs disease because they do not exhibit a severe buildup of brain GM2 or the abnormal behavioral manifestations that are seen in human Tay-Sachs patients.

Sialidases or neuraminidases (EC 3.2.1.18) are a family of *exo*-glycosidases that cleave non-reducing sialic acids that are glycosidically linked to the saccharide chains of glycoproteins, glycolipids or oligosaccharides. Sialidases have been implicated in crucial biological processes, including regulation of proliferation, control of cell adhesion, modeling of myelin, and metabolism of glycoconjugates (Monti et al., 2010). In humans, sialidases are encoded by multiple genes and they differ in terms of their optimal working pH values, kinetic properties, substrate specificities, and preference for sialic acid linkage. Based on their subcellular localization, the sialidases can be categorized as lysosomal (NEU1), cytosolic (NEU2), plasma- membrane-associated (NEU3), or lysosomal/mitochondrial or intracellular-membrane-associated (NEU4) (Miyagi and Yamaguchi, 2012). NEU1, NEU3, and NEU4, are capable of hydrolyzing sialic acid residues from gangliosides. However, the lysosomal sialidase NEU1, is part of a multi-enzyme complex with β-galactosidase and protective protein-cathepsin A (Pshezhetsky and Ashmarina, 2001) and is inactive outside of the complex, and it undergoes rapid degradation in cells deficient in cathepsin A in the genetic disease galactosialidosis (Bonten et al., 2009). NEU1 is the only mammalian sialidase that is clinically relevant with NEU1 deficiency resulting in the accumulation of sialoglycoconjugates, and extensive lysosomal vacuolization, causing excessive tissue degeneration and a pediatric neurosomatic disorder (d'Azzo et al., 1982). NEU3 is highly active toward gangliosides, and in addition to being located on the plasma membrane, it can also be found facing inward on the membranes of endosomes and lysosomes (Miyagi et al., 1999; Monti et al., 2000; Zanchetti et al., 2007; Albohy et al., 2010). The principal substrates of NEU3 are GM3 and disialogangliosides such as GD1a (Papini et al., 2004; Ha et al., 2004) and it exhibits poor activity toward GM1 or GM2, at least *in vitro* (Wang et al., 2001). NEU4 is targeted to

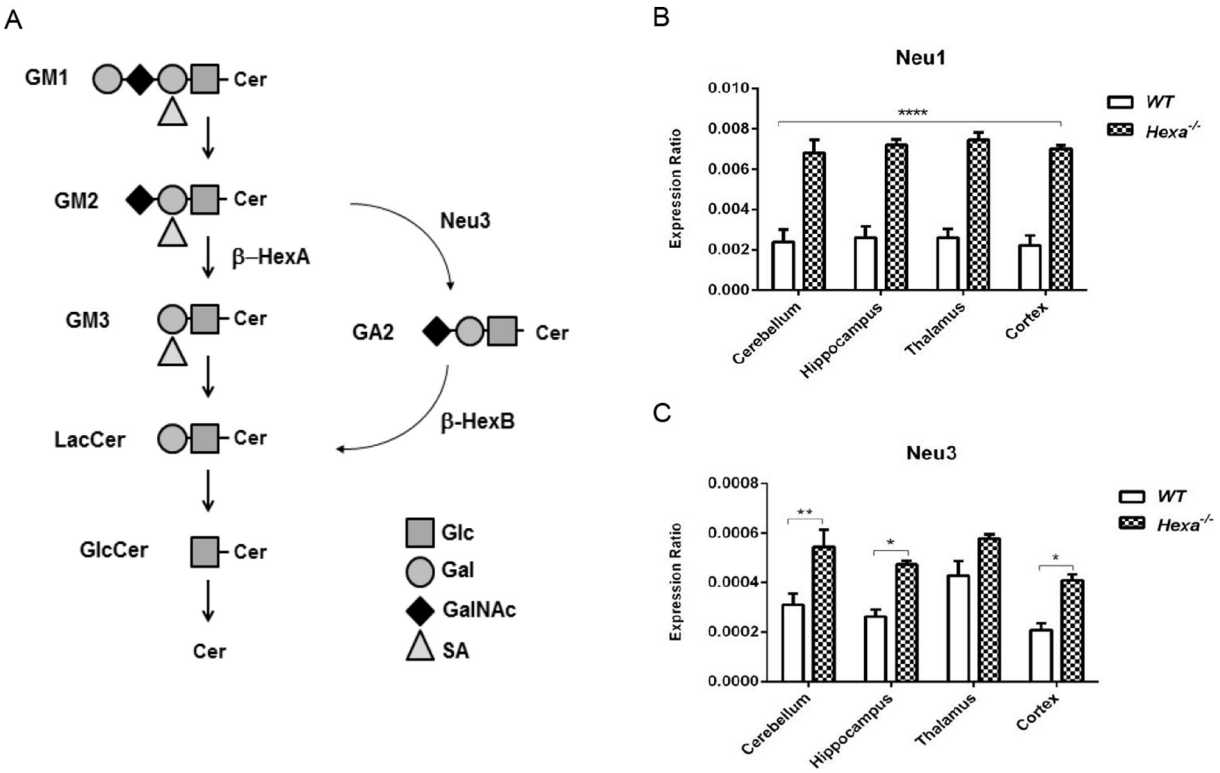

Fig. 1. (A) Schematic illustration of part of the degradation pathway of glycosphingolipids. NEU3 is the primary enzyme responsible for the metabolic bypass in the $Hexa^{-/-}$ mouse model. (B, C) Expression levels of (B) *Neu1* and (C) *Neu3* were quantified by RT-PCR for RNA isolated from four different regions of $Hexa^{-/-}$ and WT mouse brains and are represented as the ratio of the gene of interest to the *GAPDH* gene. Data represent the mean ± SE. Statistical analysis was performed using one-way ANOVA (*$p < 0.05$, **$p < 0.025$, and ****$p < 0.0001$).