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Cell cycle arrest in Batten disease lymphoblast cells

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ABSTRACT

Batten disease is an inherited neurodegenerative disorder caused by a *CLN3* gene mutation. Batten disease is characterized by blindness, seizures, cognitive decline, and early death. Although apoptotic cell death is one of the pathological hallmarks of Batten disease, little is known about the regulatory mechanism of apoptosis in this disease. Since the *CLN3* gene is suggested to be involved in the cell cycle in a yeast model, we investigated the cell cycle profile and its regulatory factors in lymphoblast cells from Batten disease patients. We found G1/G0 cell cycle arrest in Batten disease cells, with overexpression of p21, sphingosine, glucosylceramide, and sulfatide as possible cell cycle regulators.

1. Introduction

Depends on the age of onset, cellular symptoms and composition neuronal ceroid lipofuscinoses (NCLs) are designated into four different major subtypes, such as, infantile (INCL), late-infantile (LNCL), juvenile (JNCL) and adult types. At least seven different mutated genes are the underlying genetic basis of various forms of NCLs (Haltia, 2003; Mitchison and Mole, 2001; Wisniewski et al., 2001). Batten disease, also known as JNCL, is an inherited disorder with progressive neurodegeneration that usually manifests at the age of 5 to 7 years. Batten disease is caused by mutations in the *CLN3* gene (Consortium, 1995; Luberto et al., 2002). The CLN3 protein is composed of 438 amino acids and contains six putative transmembrane domains (Korkotian et al., 1999; Nugent et al., 2008). The function of CLN3 protein is not fully understood, but studies suggest that CLN3 is closely involved in apoptotic cell death (Huang et al., 1997; Luiro, 2006; Phillips et al., 2005).

The *CLN3* gene has been suggested to be involved in the cell cycle in yeast (Eckhardt et al., 2007; Guo et al., 1999) and in humans (Puranam et al., 1999; Rusyn et al., 2008). The cell cycle is controlled by cyclin-dependent kinases (CDKs) and inhibited by two groups of CDK inhibitors (Dulić et al., 2000; Jung et al., 2010). One group is the INK4 family including p15, p16, p18, and p19; the other group is the CIP/KIP family including p21, p27, and p57. p21 is a broadly acting CDK inhibitor and a determinant for the type of cell death: (Hait et al., 2009; Harper et al., 1993) apoptosis or autophagy (Fujiwara et al., 2008; Osório et al., 2007). p21 is a negative regulator that maintains cells in G_0 when the conditions for cell proliferation are not optimal (Jung et al., 2010). Changes in cell cycle-related protein expression were reported in the *Ppt1*$^{-/-}$ and *Cln6*^{nclf} mouse models (Kielar et al., 2009). However, little is known about the detailed cell cycle profile in Batten disease.

CLN3-deficient cells have high rates of apoptosis and altered sphingolipid levels (Rusyn et al., 2008). Mutant CLN3 protein is incapable of binding to GalCer and lacks the ability to modulate sphingolipid metabolism (Dulić et al., 2000; Persaud-Sawin and Boustany, 2005; Vermeulen et al., 2003). Sphingolipids are composed of three main types: sphingomyelins, glycosphingolipids, and gangliosides (Fuller, 2010; Hait et al., 2009). Sphingolipid metabolites are not merely structural components but are also regulators of cell survival, growth, and movement (Benedict et al., 2007; Osório et al., 2007; Spiegel and Milstien, 2003; Tuxworth et al., 2011; Wei et al., 2007).

Sphingosine is a ligand for steroidogenic factor 1. When phosphorylated as sphingosine-1-phosphate (S1P), it modulates histone acetylation and p21 expression (Lucki and Sewer, 2012). Sphingosine itself

Abbreviations: NCL, neuronal ceroid lipofuscinosis; CDK, cyclin-dependent kinase; S1P, sphingosine-1-phosphate; GlcCer, glucosylceramide; TLC, thin layer chromatography; FA, fact acid; LacCer, lactosylceramide; Sulf, sulfatide; So, sphingosine.

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can induce cell death. The amount of intracellular sphingosine is regulated by enzymes that are ceramidases, ceramide synthases, and sphingosine kinases (Lucki and Sewer, 2012).

Glucosylceramide is a glycolipid containing a fatty acid, glucose, and sphingosine. Glucosylceramide is essential for sustaining growth of hippocampal neurons (Luberto et al., 2002). High amounts of glucosylceramide induce increased endoplasmic reticulum density and functional calcium stores in cultured neurons (Korkotian et al., 1999).

Sulfatide is a sulfated galactosylceramide that is synthesized primarily in oligodendrocytes (Huang et al., 1997). The accumulation of sulfatide is associated with progressive demyelination and various lethal neurological syndromes (Eckhardt et al., 2007). CLN3 protein binds to sulfatide but its biological role in Batten disease is still obscure (Rusyn et al., 2008).

In this study, we investigated the cell cycle profile of Batten disease lymphoblasts and monitored the expression of p21 and several sphingolipids that might be related to the cell cycle and to Batten disease.

2. Materials and methods

2.1. Cell culture

Normal lymphoblast cells and lymphoblast cells from Batten disease patients were obtained at the Coriell Institute for Medical Research (Cat. number GM08794 and GM08820). Cells were cultured on 100 mm culture plates in Roswell Park Memorial Institute 1640 medium (RPMI1640; Caisson, Utah, USA) with 10% fetal bovine serum (FBS; GemCell, California, USA) and 1% penicillin from solution (Invitrogen, California, USA) at 37 °C in an incubator containing 95% air and 5% CO_2 (Thermo Scientific, Massachusetts, USA).

2.2. MTT assay

Changes in cell viability were evaluated using an MTT assay. Lymphoblast cells from Batten disease patients and normal lymphoblast cells were cultured in 96-well plates (10^3 cells/well) containing 100 μl of medium, and incubated for 24 h at 37 °C in a CO_2 incubator. An aliquot of 10 μl of EZ-cytox MTT assay solution (Daeil Lab Service, South Korea) was added to each culture medium and incubated for 2 h in a CO_2 incubator. After incubation, the optical density was measured at 450 nm.

2.3. Western blot

Approximately 1×10^6 CLN3 lymphoblast cells were cultured on 100 mm plates in culture medium on 37 °C in a CO_2 incubator. Cells were collected and proteins were extracted using modified radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris, 150 mM NaCl, 0.1% SDS, 0.5% sodium deoxycholate, 1% Nonidet P-40) with a protease-inhibitor cocktail (Sigma, USA). From each sample, 30 μg of protein was measured by BCA assay kit (Thermo, USA), electrophoresed

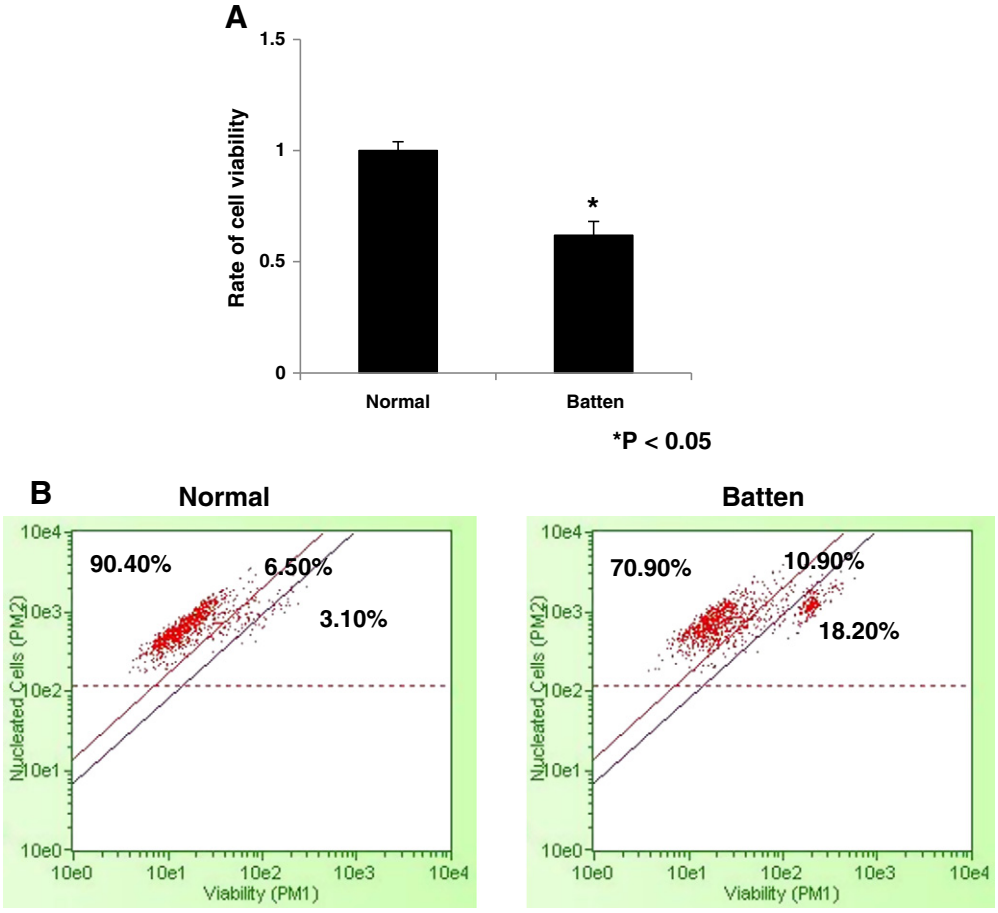

Fig. 1. Measurement of viability and apoptosis in Batten disease and normal cells. (A) Batten disease cells showed decreased cell viability. MTT assay was performed as described in the Materials and methods. Data were normalized to the viability of normal cells. Mean values of triplicate samples with SD are shown. Significance was determined using Student's *t*-test (*$p < 0.05$). (B) Batten disease cells showed increased apoptosis and death rates. Batten disease and normal cells were treated with ViaCount reagent and were run on a Guava EasyCyte flow cytometer. Left of red marker, live cells; center of dot plot between red and purple marker, apoptotic cells. Right of purple marker, dead cells.