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Research Paper

Chitinase-3-like Protein 1: A Progranulin Downstream Molecule and Potential Biomarker for Gaucher Disease

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ABSTRACT

We recently reported that progranulin (PGRN) is a novel regulator of glucocerebrosidase and its deficiency associates with Gaucher Diseases (GD) (Jian et al., 2016a; Jian et al., 2018). To isolate the relevant downstream molecules, we performed a whole genome microarray and mass spectrometry analysis, which led to the isolation of Chitinase-3-like-1 (CHI3L1) as one of the up-regulated genes in PGRN null mice. Elevated levels of CHI3L1 were confirmed by immunoblotting and immunohistochemistry. In contrast, treatment with recombinant Pcgin, a derivative of PGRN, as well as imiglucerase, significantly reduced the expressions of CHI3L1 in both PGRN null GD model and the fibroblasts from GD patients. Serum levels of CHIT1, a clinical biomarker for GD, were significantly higher in GD patients than healthy controls (51.16 ± 2.824 ng/ml vs 35.07 ± 2.099 ng/ml, $p < 0.001$). Similar to CHIT1, serum CHI3L1 was also significantly increased in GD patients compared with healthy controls (1736 ± 152.1 pg/ml vs 684.7 ± 68.20 pg/ml, $p < 0.001$). Whereas the PGRN level is significantly reduced in GD patients as compared to the healthy control (91.56 ± 3.986 ng/ml vs 150.6 ± 4.501, $p < 0.001$). Collectively, these results indicate that CHI3L1 may be a previously unrecognized biomarker for diagnosing GD and for evaluating the therapeutic effects of new GD drug(s).

1. Introduction

Gaucher disease (GD), common lysosomal storage disease (LSD), is caused by mutations in *GBA1* with resultant defective glucocerebrosidase (GCase) activity and consequent accumulation of its substrate β-glucosylceramide (β-GlcCer) in macrophages and other cell types (Jian et al., 2016a). There are three types of GD based on its primary central nervous system (CNS) involvement: Type 1 does not manifest with early onset primary CNS disease and has previously been described as non-neuropathic. Patients with GD types 2 and 3 have primary CNS impairments. Type 2 features acute neuropathic disease of infancy. Type 3 is marked by chronic neuropathy with highly variable primary CNS onset and involvement. The peripheral manifestations of GD include hepatosplenomegaly and pancytopenia, due to bone marrow infiltration and splenic sequestration. Although *GBA1* mutations are the primary cause of GD, there is broad heterogeneity in clinical manifestations even among patients carrying the same *GBA1* mutations, ranging from very early disease onset to very mild clinical presentations (Biegstraaten et al., 2011; Elstein et al., 2010). These diverse variations are not directly attributable to different *GBA1* mutations and may relate to unidentified modifier genes.

PGRN is also known as granulin–epithelin precursor (GEP) (Zanocco-Marani et al., 1999), proepithelin (PEPI) (Shoyab et al., 1990; Plowman et al., 1992), acrogranin (Anakwe and Gerton, 1990), and GP88/PC-cell derived growth factor (PCDGF) (Zhou et al., 1993). PGRN is a growth factor with multiple functions, including promoting cell proliferation, stimulating wound healing (Zhao and Bateman, 2015; He et al., 2003) and regulating immune response (Fu et al., 2016; Jian et al., 2013a; Jian et al., 2018; Liu et al., 2014; Liu and Bosch, 2012; Mundra et al., 2016; Wei et al., 2014a; Wei et al., 2016; Williams et al., 2016). PGRN is also an anti-inflammatory molecule that directly binds to TNFR, inhibits TNFα/TNFR1 inflammatory signaling (Liu and Bosch, 2012; Jian et al., 2013b; Liu, 2011; Tian et al., 2014;

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Tian et al., 2012; Zhao et al., 2013a; Tang et al., 2011), and activates the TNFR2 anti-inflammatory pathway (Zhao et al., 2015; Zhao et al., 2013b; Wei et al., 2014b; Li et al., 2014; Fu et al., 2017). Autoantibodies against PGRN have been detected in sera from patients with colitis, rheumatoid arthritis, and other autoimmune diseases, and these antibodies block PGRN binding to TNFR (Thurner et al., 2013a; Thurner et al., 2015; Thurner et al., 2014; Thurner et al., 2013b). Insufficiency of PGRN also associates with various types of CNS diseases, including frontotemporal lobe dementia (haploinsufficiency) (Baker et al., 2006; Cruts et al., 2006), Alzheimer disease (Brouwers et al., 2008), and Parkinson disease (Hu et al., 2006; Brouwers et al., 2007). In addition, intracellular PGRN plays an important role in lysosome biology, and homozygous deficiency of PGRN causes lysosomal storage diseases, including neuronal ceroid lipofuscinosis (Ahmed et al., 2010; Smith et al., 2012) and Gaucher-like disease (Jian et al., 2016a; Choy and Christensen, 2016; Jian et al., 2016b). Serum levels of PGRN are significantly decreased in GD patients, and *GRN* variants were found to be prevalent in GD patients. PGRN-deficient mice develop a typical GD-like phenotype. Mechanistically, PGRN functions as a chaperone that facilitates the lysosomal delivery of GCase, the defective enzyme in GD (Jian et al., 2016a; Jian et al., 2016b). This function of PGRN may be operative with other lysosome enzymes (Jian et al., 2017). Interestingly, PGRN and a PGRN-derived peptide, Pcgin, ameliorated GD phenotypes in mouse models and fibroblasts obtained from GD patients.

Chitinases (EC 3.2.2.14) are hydrolytic enzymes that break down glycosidic bonds in chitin (Rathore and Gupta, 2015). They belong to 18 glycosyl hydrolase family, an ancient gene family that is widely expressed from prokaryotes to eukaryotes. Chitotriosidase (CHIT1) was the first discovered and characterized mammalian chitinase (Boot et al., 1998). CHIT1 has been used as a biomarker for lysosomal storage diseases, including GD (Hollak et al., 1994; Wajner et al., 2004; van Dussen et al., 2014) and Niemann Pick diseases (Wajner et al., 2004). CHIT1 protein and enzymatic activity levels decrease with enzyme replacement therapy in GD patients (Drugan et al., 2017). Chitinase-3-like protein 1 (CHI3L1), also called YLK-40 (based on its three N-terminal amino acids: tyrosine (Y), lysine (K), and leucine (L)), is a 40 kDa mammalian glycoprotein. CHI3L1 binds chitin polymers but lacks chitinase activity. CHI3L1 is a pro-inflammatory marker and elevations of CHI3L1 have been found in auto-immune diseases, such as rheumatoid arthritis, pulmonary sarcoidosis (Di Rosa et al., 2016), psoriasis (Baran et al., 2017), interstitial lung disease (Hozumi et al., 2017), hypertension (Xu et al., 2017), and various types of cancer (Erturk et al., 2017; Kotowicz et al., 2017; Shaker and Helmy, 2017; Wan et al., 2017). CHI3L1 binds to IL-13 receptor α2, and exerts its biological function by activating downstream mitogen-activated protein kinase, protein kinase B/AKT, and Wnt/β-catenin signaling (He et al., 2013). In this study CHI3L1 was identified as a novel downstream molecule that has potential to be employed as a novel biomarker for diagnosing GD and for examining the therapeutic effects of new GD drug(s).

2. Material and Methods

2.1. Reagents

Fibroblasts from GD patients with L444P homozygosity were purchased from Coriell Cell Repositories (Camden, NJ) and cells were cultured in DMEM supplemented with 10% FBS. Antibodies against CHI3L1 and GAPDH were purchased from Santa Cruz Biotechnology (Dallas, Texas).

2.2. Gene Expression Analysis

C57BL/6 WT mice and PGRN KO mice were maintained at NYU Animal Facility in accordance with an Institutional Animal Care and Use Committee approved protocol. Comparative gene array analysis between WT and PGRN KO CD4+ T cells has been described previously (Mundra et al., 2016). Briefly, naïve $CD4^+$ T cells were isolated from spleen of WT and PGRN KO mice, and activated using CD3 and CD28 antibody (Ab) over 24 h. RNA was extracted and quantified with NanoDrop ND-1000. RNA integrity was assessed by standard denaturing agarose gel electrophoresis. The Mouse 4 × 44K Gene Expression Array v2 (Agilent Technology) with about 39,000+ mouse genes and transcripts represented, all with public domain annotations, was employed for genomic profiling. Sample labeling and array hybridization were performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technology). Agilent Feature Extraction software (version 11.0.1.1) was used to analyze the acquired array images. Quantile normalization and subsequent data processing were performed with using the GeneSpring GX v11.5.1 software (Agilent Technologies). Differentially expressed genes were identified through Fold Change filtering.

2.3. Real-time Quantitative PCR

Various tissues, including spleen and lung, from WT and PGRN KO mice were dissected and homogenized in TRIzol® reagent. RNA extraction was performed in accordance with the manufacturer's recommended protocol. cDNA was synthesized using SuperScript® Reverse Transcriptase (Invitrogen). SYBR® Green PCR Master Mix (Applied Biosystems) was employed in real-time PCR and the reaction was performed with StepOnePlus™ Real-Time PCR Systems (Applied Biosystems). The mRNA levels of target genes were normalized to GAPDH. The following sequence-specific primers were used for the real-time qPCR: 5′- ACGATTTCCATGGA GTCTGG-3′ and 5′- AATCCTTCCCCTGAGATTGG -3′ for mouse CHI3L1, 5′-CCAGCATATG GGCATACCTT-3′ and 5′-CAGACCTCAGTGGCTCCTTC -3′ for mouse CHI3L3, 5′-CAGTGG CTCAAGGACAACAA-3′, 5′-CGTGGAAAAACCGTTGAAGT-3′ mouse CHI3L4. The presence of a single specific PCR product was verified by melting curve analysis and the experiments were repeated three times.

2.4. GD Mouse Model

The induction of the GD phenotype in PGRN null mice has been described (Jian et al., 2016a; Jian et al., 2016b). Briefly, the GD phenotype was induced in 8 week-old C57B/L6 WT and PGRN KO mice by (intraperitoneal) IP injection of OVA-Alum (Aluminium hydroxide-emulsified Ovalbumin) at Day 1 and Day 15, followed by intranasal challenge of 1% OVA beginning at Day 29 at a frequency of three times per week for four weeks. In rescue experiments, 4 mg/kg recombinant PGRN or Pcgin, or 60 U/kg imiglucerase were IP injected weekly following initiation of intranasal challenge. Spleens, livers, limbs, and lungs were collected at sacrifice.

D409V/- GD mouse is a model generated by disruption of one *Gba1* allele, and point mutation, D409V, on the other allele (Barnes et al., 2014). The D409V GCase is unstable and rapidly degraded (Liou et al., 2006) and the D409V/- mice develop Gaucher cells at around 8 weeks of age. At 5-weeks-old, D409V/- mice were injected with rPGRN (4 mg/kg/week) for 4 weeks and then sacrificed for examination of CHI3L1 expression from spleen lysate ($n = 6$ per group).

2.5. Isolation of Organelle From Spleen Tissues

Spleens from WT and PGRN KO mice with OVA challenge were dissected and the cellular organelles were isolated by using a modified protocol of the Lysosome Isolation Kit (Sigma-Aldrich). Briefly, spleen tissues were homogenized in 1 mL 1× Extraction buffer (provided by the kit) and centrifuged at 1,000 ×*g* for 10 min at 4 °C. The upper lipid layer was removed and the supernatant was transferred to a sterile Eppendorf tube. Another 1 mL Extraction buffer was added to the pellet and the homogenization was repeated. Pellets containing nuclei and cellular debris were discarded and supernatant was centrifuged at

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