New macrophage models of Gaucher disease offer new tools for drug development

Daniel K. Borger, Ellen Sidransky, Elma Aflaki

Section of Molecular Neurogenetics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, 20892, USA

Correspondence: Ellen Sidransky
E-mail: sidranse@mail.nih.gov
Received: March 12, 2015
Published online: March 29, 2015

Gaucher disease is an inherited enzyme deficiency resulting in the lysosomal accumulation of specific glycolipids in macrophages and, in some cases, neurons. While current treatments are effective at reducing this glycolipid storage in macrophages, they are expensive and ineffective in treating neurological manifestations of the disease, driving the search for novel therapeutics. Moreover, mutations in GBA1, the gene implicated in Gaucher disease, are an important risk factor for the development of Parkinson disease and related disorders, an association that has further heightened interest in Gaucher disease research. However, the development of therapeutic strategies has been hampered by a shortage of appropriate cellular models of Gaucher disease. We have generated two novel macrophage models of Gaucher disease, one through the differentiation of peripheral blood monocytes from patients with Gaucher disease and the other through the differentiation of induced pluripotent stem cells derived from patient fibroblasts. Both disease models demonstrate similar cellular phenotypes and exhibit extensive glycolipid storage when exposed to exogenous lipid sources such as erythrocyte membranes. Furthermore, we have used these models to confirm the efficacy of a novel small molecule in clearing glycolipid storage and restoring normal macrophage function. These results demonstrate the usefulness of these models in exploring new therapeutics for Gaucher disease and related disorders.


Gaucher disease (GD) is a lysosomal storage disease caused by mutations in the GBA1 gene which leads to deficiency of the lysosomal enzyme glucocerebrosidase. In GD, partial or complete loss of glucocerebrosidase activity causes the buildup of the glycolipids glucosylceramide and glucosylsphingosine in the lysosomes of macrophages. This buildup leads to the formation of glycolipid-engorged Gaucher macrophages (GMs), often referred to as “Gaucher cells,” which are typically filled with periodic acid–Schiff stain (PAS) positive glycolipid deposits that displace the nucleus and distort the normally spherical lysosomal architecture. These GMs accumulate in various tissues associated with the reticuloendothelial system and are a classic finding in GD [1]. The disruption of cellular homeostasis due to glycolipid accumulation is thought to prompt GMs to secrete inflammatory cytokines, and both glycolipid storage and cytokine secretion likely contribute to the organomegaly, cytopenia, and bone disease that are common in patients with GD [2,3,4]. Lipid accumulation in the central nervous system can also result in neuronopathic symptoms; hence GD is divided into non-neuronopathic (type 1 GD) and neuronopathic (type 2 GD and type 3 GD) forms. Furthermore, homozygosity and heterozygosity for mutations in GBA1 have been found to be more frequent in patients with Parkinson disease and related Lewy body disorders. In fact, mutations in GBA1 are now considered the most common known genetic risk factor for Parkinson disease [5,6].
Gaucher disease is commonly treated by enzyme replacement therapy, where recombinant glucocerebrosidase is infused intravenously to patients on a regular basis. While this therapy is effective for patients with type 1 GD, it is extremely expensive. Furthermore, the inability of recombinant glucocerebrosidase to pass through the blood-brain barrier means enzyme replacement is ineffective at treating neuronopathic manifestations of the disease. These factors, along with the discovery of a link between Parkinson disease and GD, have driven an expanding search for alternative drugs aimed at augmenting endogenous glucocerebrosidase activity. However, studies regarding both potential treatments and the pathogenesis of GD have long been impeded by the lack of appropriate cell-based models exhibiting glycolipid storage analogous to that seen in patient macrophages. While research in the field has been performed using patient fibroblasts, these cells do not store the implicated lipids. Recently, we and others have set out to generate more appropriate cell-based models of GD, with a particular focus on macrophages.

We first developed a macrophage model for GD by differentiating primary human macrophages from CD16^+ peripheral blood monocytes isolated from 20 patients with various GBA1 mutations. While our results using these cells have proved promising, the use of primary cells has several disadvantages. The amount of blood required renders it difficult or impossible to obtain adequate numbers of cells from infants with the most severe forms of GD. Furthermore, a ready source of blood is required for these studies, as neither monocytes nor differentiated macrophages can be propagated in culture. One potential solution to this latter requirement would be to generate human macrophage cell lines with wild-type and mutant GBA1 genes by immortalizing primary peripheral blood monocyte-derived macrophages. However, immortalization of differentiated human macrophages poses a number of problems - in particular the difficulty of stably transfecting primary macrophages - and to our knowledge it has yet to be attempted successfully.

In our recent publication, we aimed to overcome these issues by generating induced pluripotent stem cells (iPSCs) from fibroblasts of patients with both type 1 and type 2 GD [7]. While generating and maintaining iPSCs is both labor-intensive and expensive, fibroblasts can readily be obtained in sufficient numbers from young patients with type 2 GD, and the resulting iPSCs can be propagated in culture. We used lentiviral infection to induce expression of the four "Yamanaka factors" (Oct4, Sox2, cMYC, and KLF4) necessary to induce pluripotency in cultured patient fibroblasts. The pluripotency of these cells was confirmed based on their expression of several markers and their ability to generate teratomas in all three germ layers in mice. We then differentiated these cells, first into CD14^+ monocytes, and then into CD68^+ macrophages (Figure 1).

We next determined whether iPSC-derived GMs were able to recapitulate the glycolipid storage and enzymatic deficiencies of GMs observed in vivo. Western blotting and enzyme activity assays established that iPSC-derived GMs had lower glucocerebrosidase protein levels and enzymatic activity than control macrophages. The GMs, but not control macrophages, also exhibited limited glucosylceramide storage
under normal culture conditions as determined by liquid chromatography-mass spectrometry. However, they did not demonstrate the massive glycolipid storage observed in GMs in vivo.

We hypothesized that this limited storage was a result of cells in culture not being exposed to a constant source of lipids. Tissue-resident macrophages are responsible for the clearance of aged and dying cells whose cells membranes provide large amounts of lipids. To emulate this situation in culture, we incubated both iPSC-derived and primary macrophages with lipid-rich erythrocyte ghosts prepared from GD patient blood samples (Figure 1). The use of erythrocyte ghosts from patients with GD is particularly appropriate for these studies, as the membranes of erythrocytes in GD are known to contain elevated levels of the implicated glycolipids [8]. Incorporating fluorescently tagged glucosylceramide into these erythrocyte ghosts enabled the evaluation of glycolipid storage by fluorescence microscopy. When fed with these erythrocytes, GMs demonstrated much higher levels of glucosylceramide and glucosylsphingosine, while control macrophages were able to effectively catabolize the phagocytosed glycolipids.

Various other elements of macrophage function were also evaluated in these cellular models. We found that GMs could efficiently phagocytize both opsonized whole erythrocytes and E. coli, but that they exhibited impaired chemotaxis in response to CCL3, CCL5, and CXCL12, all potent leukocyte chemoattractants. Moreover, GMs exhibited reduced intracellular reactive oxygen species production. This appeared to contribute to defective digestion of phagocytosed material, providing new insights into defective macrophage function in GD.

In these experiments, we included blood and skin biopsies from 18 patients with type 1 Gaucher disease who shared the common GD genotype N370S/N370S. This enabled us to compare primary macrophage lines to iPSC-derived macrophages, all with the same GD genotype. In fact, in some cases we had both primary macrophages and iPSC-derived macrophages from the same donor, providing a powerful tool to confirm the validity of results from iPSC-derived GMs. Both macrophage models recapitulated the disease features of reduced enzymatic activity and glycolipid storage, and both behaved similarly in lipid studies and functional assays. These models can therefore serve as an important tool in drug and biomarker discovery and validation, vital steps in the development of new therapeutics and diagnostics for patients with GD and related disorders.

We used this GM model to validate the efficacy of a novel non-inhibitory glucocerebrosidase chaperone molecule, which was previously identified via high throughput screening. In this screening process, we used mutant glucocerebrosidase (N370S) from patient tissue extracts to evaluate the effects of a large number of small molecules on glucocerebrosidase enzymatic activity, allowing us to determine the effect of these compounds on the mutant enzyme while in the presence of any necessary cofactors [9]. Screening in this way, we were for the first time able to identify molecules that enhanced the enzymatic activity of human glucocerebrosidase. We chose one lead compound class, NCGC758, to validate in the GM models. In both primary and iPSC-derived GMs, immunocytochemistry and liquid chromatography-mass spectrometry of stored lipid species indicated that NCGC758 enhanced glucocerebrosidase activity and reduced lipid storage. In addition, administration of the compound to GMs in culture normalized chemotaxis and phagocytic indexes.

Several other groups have also evaluated patient iPSC-derived macrophages in culture. Tiscornia et al. derived iPSCs from fibroblasts of a patient with type 2 GD that exhibited severe enzyme deficiency [9]. They used lentiviral infection with a functional GBA1 gene to generate an isogenic iPSC line with restored glucocerebrosidase function. They showed that iPSC lines with and without normal levels of glucocerebrosidase enzymatic activity were both able to be effectively differentiated into macrophages, indicating that GBA1 mutations do not interfere with the in vitro differentiation process.

Panicker et al. derived iPSC lines from patients with all three forms of GD [11,12]. They also demonstrated defective phagocytosis in these iPSC-derived GMs [11], and more recently showed that these cells exhibit greatly increased secretion of the pro-inflammatory cytokines tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 in response to lipopolysaccharide (LPS) treatment [12]. These results reflect the increased expression of these cytokines observed in some patients with type 1 GD [2,4]. Further studies in these GM models may assist in elucidating the link between glucocerebrosidase deficiency and the pro-inflammatory activity of macrophages in GD.

Thus, both our lead compound and new macrophage models can be used to accelerate research into both the etiology of GD and the discovery of novel therapeutics. Moreover, since deficient glucocerebrosidase is implicated in the pathogenesis of Parkinson disease and related disorders, further development of such enzyme-enhancing therapies could have implications for the treatment of different forms of parkinsonism, thus extending the relevance of our findings far beyond GD.
Acknowledgements

This work was supported by the Intramural Research Programs of the National Human Genome Research Institute, and the National Institutes of Health.

References