Mechanisms of Gaucher disease pathogenesis

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What is Gaucher disease?

Gaucher disease is caused by mutations in the *Gba1* gene encoding an acid β-glucocerebrosidase (GBA1), the lysosomal hydrolase which breaks down glucosylceramide (GlcCer). In Gaucher type 1 disease the accumulation of this simple glycolipid is mainly restricted to tissue phagocyte lysosomes resulting ultimately in hepatomegaly, splenomegaly and osteopenia. Lower residual GBA1 levels leads to neuronal storage, in types 2 and 3 neurological symptoms are characterised by acute (death at age 2) or sub-acute onset, respectively. The links between cellular changes and clinical manifestations are largely unknown but are the key to the development and monitoring of new therapies.

The newcomer to Gaucher disease is likely attracted to the apparent simplicity of an autosomal recessive disorder which promises to unravel the critical GlcCer function in normal cells (GlcCer is widespread, it's even present in some bacteria—also, mouse and fly GlcCer knockouts die at embryo stage). However, closer acquaintance reveals not a classic Mendelian disorder—sometimes even monozygotic twins have different symptoms—and studies at the cellular level have so far failed to reveal clear GlcCer functions. Now a team led by Ellen Sidransky at the NIH has taken what appears to be a big step forward by producing two *in vitro* models of Gaucher cells (1).

How has Gaucher disease been investigated?

Research has been hampered by the inaccessibility of Gaucher macrophages and the lack of *in vitro* models. The simplest approach has been to induce a Gaucher phenotype by treating cells with the GBA1 inhibitor, conditurol-β-epoxide. Whilst this method has the virtues of being cheap and experimentally easy, off-target effects are not controlled for. For instance, conduritol-β-epoxide also inhibits a related enzyme, GBA2 (2). Inhibition of this non-lysosomal enzyme has been reported to rescue mutations in lysosomal GBA1 (3). GlcCer is unusual amongst glycolipids with intracellular trafficking connecting both lysosomal and non-lysosomal pools on both sides of the bilayer membrane (4). GlcCer transporters have been identified (5-7) but the relationships between different pools of GlcCer are still unclear.

A second approach has been to use fibroblasts from Gaucher patients. Although the macrophage-centric view of Gaucher disease has recently been questioned (8), Gaucher skin fibroblasts are not important in Gaucher disease and don't store GlcCer.

What has now been achieved?

The researchers selected 20 Gaucher patients representing a total of 4 genotypes. Monocytes were extracted from these patients and differentiated into macrophages by the use of M-CSF: the resulting cells being termed hMacs. This method, whilst relatively quick and cheap, does not lead to a sustainable cell line. This was addressed by taking Gaucher fibroblasts from 4 patients and transformed them into induced pluripotent stem cells (iPSCs), then to monocytes and finally macrophages, referred to as iMacs. Whilst expensive and difficult, the use of stem cell technology means that this method does generate a sustainable cell line. Control cells of both types were produced from blood and fibroblasts donated by healthy volunteers.

Researchers examined the two cell types produced, hand-in-hand with an evaluation of previously disclosed (9) prototype drug NCGC00188758. This belongs to the class of molecules

Page 2 of 3

Wheeler and Sillence. New developments in Gaucher disease

known as molecular chaperones: binding to the enzyme (in this case GBA1) correcting the misfolding. This results in repaired transport to the lysosome and enhanced GBA1 function.

Gratifyingly the phenotypes of hMacs and iMacs resembled genuine Gaucher cells. Compared with control macrophages, both cell types showed reduced impaired GBA1 activity and impaired transport of mutant GBA1 to lysosomes, indicated by colocalisation with the lysosomal marker LAMP2. Crucially, and in marked contrast to Gaucher fibroblasts, both cell types accumulated GlcCer and glucosylsphingosine. Cellular defects were rescued by treatment with NCGC00188758. Indeed, this small molecule drug was slightly more effective at restoring GBA1 activity than imiglucerase, an enzyme commonly used in enzyme replacement therapy.

Chemotaxis was found to be reduced versus controls, an observation previously reported for some, though not all, Gaucher patients (10). Whilst the Gaucher model cells were found to phagocytose IgG-opsonised erythrocytes and bacteria normally, dysfunction was found in the production of reactive oxygen species (ROS). Thus iMacs and hMacs had lower concentration of ROS in the resting state, and no further generation of ROS upon phagocytosis. These findings mirror previous reports on impaired superoxide generation in Gaucher cells (10,11). Importantly both chemotaxis and ROS production were restored on treatment with NCGC00188758.

Gaucher links with Parkinson's disease

The potential medical significance of Gaucher disease does not end with the condition itself. Most attention has been focussed on the unexpected finding that having even one mutant copy of the Gba1 gene is a significant risk factor for Parkinson's disease (12). This has prompted research interest into the possible links between Gaucher disease and Parkinson's disease. The most fundamental observation is that poorly functional GBA1 is associated with the accumulation of α-synuclein (α-syn) (13) leading to neuronal death. This protein can fold and aggregate in many different ways and a possible mechanism for its accumulation is the stabilisation of oligomers by GlcCer (13). In turn, α-syn can inhibit GBA1 (14), an observation that may well account for the reduced levels of GBA1 activity seen in post mortem brains of sporadic Parkinson's disease patients (15). Furthermore, α-syn can interfere with vesicular traffic of GBA1 from the ER to the Golgi (13). Thus, by means of a bi-directional

loop, even a slight loss of GBA1 function can become magnified. A qualification to the above discussion arises from the observation that post-mortem brains of patients suffering from all types of Gaucher disease had monomeric, but not oligomeric α-syn (16). Further work is needed to unravel the exact mechanism by which mutant Gba1 gives rise to α-syn aggregates. Further explorations of the consequences of this accumulation of cytosolic, insoluble α-syn are also required. It has been shown, for example, that in normal neurones α-syn is localised at the synaptic membrane [where it plays a role in regulating synaptic vesicles (17)] and that this localisation is mediated by lipid rafts (18). How these changes relate to increased raft-forming GlcCer has yet to be addressed.

Future research using Gaucher cell models

Recent research has revealed interdependence of phagosome pH and ROS generation (19) hence decreased generation of ROS might be linked to increased pH of Gaucher lysosomes (20). Increased pH may also explain reduced lysosomal proteolysis (13), co-storage of cholesterol and disrupted membrane trafficking in Gaucher cells (21). Alternatively, glucosylsphingosine (GlcSph) may mediate decreased ROS (22). Whilst interest has generally focussed on GlcSph as a biomarker for Gaucher disease, it's still an open question whether enough GlcSph escapes the lysosome to inhibit Protein kinase C [IC_{50} =85 μM (23)]. However, PKC has also been implicated in the phagocytosis of opsonised bacteria (24).

Several workers have reported increased levels of inflammatory markers, including M-CSF, in the serum of patients with Gaucher disease. These observations raise the possibility that this could be the cause of the reported proliferation of osteoclasts associated with Gaucher disease (25,26) and the consequent occurrence of bone symptoms in some patients.

In conclusion it appears that the researchers have produced both a realistic model of Gaucher cells and a promising prototype drug. Although much work is required before NCGC00188758 can be considered as a usable drug in patients, there is a particular lack of treatment options for the neurodegenerative forms of Gaucher disease (12,27).

Acknowledgements

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