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New LSD Therapies Unfolding

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NIH researchers report results of a qHTS hunt for new chemical chaperones for the treatment of Gaucher disease [1].

Lysosomes are cellular recycling centers. They are sac-like organelles containing largely hydrolytic enzymes that operate in the low pH environment and are responsible for the turnover of cellular constituents within to simple products for building new molecules. The construction of the lysosome and its enzymes is a highly orchestrated sequence of biosynthetic, targeting, folding, and trafficking events that take place in the endoplasmic reticulum and Golgi complex [2]. The resulting lysosomes have been engineered to maintain a kinetic balance of macromolecular recycling for optimal cell function. A large array of mutations can disrupt the enzymatic status of the lysosome, creating an imbalance that results in the detrimental accumulation of specific macromolecules. Over 40 such lysosomal storage diseases (LSDs) have been characterized in humans and are usually categorized biochemically by accumulated metabolite [3].

Gaucher disease is an LSD characterized by an accumulation of the sphingolipid glucocerebroside due to a functional deficiency of glucocerebrosidase (GC; aka acid β-glucosidase). Three types of Gaucher disease exist differentiated by the degree of neuronopathology. Symptoms range from splenomegaly, anemia, and bone lesions to progressive neurological deterioration and death. Enzyme replacement therapy is costly and is not effective in the treatment of CNS symptoms. More than 200 missense mutations can lead to Gaucher disease; they are widely distributed throughout the protein and vary in their frequency and in the severity of the resulting disease [4] (Figure 1). Most of these result in misfolding, decreased stability, and/or mistrafficking of GC to the lysosome [5]. In vitro enzyme activity of GC mutants does not explain the phenotypic diversity of the disease and the instability of many variants has led to controversy regarding their activity [6, 7].

The insight underlying the recent NIH work [1] was provided by the work of Kelly and colleagues at Scripps in 2002 [8]. Previous work had demonstrated that chemical chaperones could induce proper folding and stabilize proteins in the secretory pathway [9]. The Scripps team posited that some catalytically active, albeit unstable, GC mutants might be deficient in their ability to fold properly or at a kinetically acceptable rate that permits their effective translocation into the ER. They might be targeted for proteosomal degradation preventing their trafficking to the lysosome at levels sufficient to prevent accumulation of the sphingolipid substrate. Using cells homozygous for the prevalent N370S mutant of GC [10], the Scripps team demonstrated that analogs of the glucosidase inhibitor deoxynojirimycin, an iminosugar found in mulberry plants, at subinhibitory concentrations resulted in up to a 2-fold increase in the GC activity of treated cells. The data were consistent with the inhibitor acting as a chemical chaperone to facilitate the proper folding of the GC variant, leading to more enzyme successfully trafficked to the lysosome. Additional work has demonstrated the generality of the iminosugar chaperone approach with some, but not all, GC variants and confirmed increased GC trafficking to the lysosome [11, 12]. Although the iminosugars studied are less-than-promising drug candidates, it was clear that attempts to identify more drug-like chemical chaperones for the treatment of Gaucher disease would soon follow.

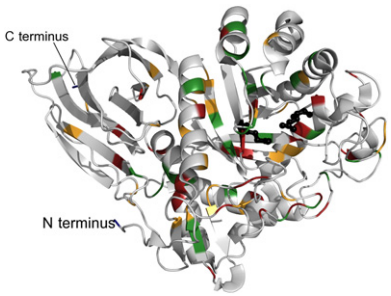

Figure 1. Mutations in GC Leading to Gaucher Disease
This figure is adopted from [4]. Position of previously identified point mutations is mapped onto the ribbon representation of GC structure. Residues are color coded as follows: dark red, residues whose mutation causes severe form of the disease; bright orange, residues whose mutation causes mild forms of the disease; in yellow and dark green, residues that are found mutated but without clinical data regarding the severity of the disease. The key active site residues, E235 and E340, are shown in black and are not mutated in the disease. Figure created using PyMOL software (http://www.pymol.org).

The recent NIH screening effort [1] showcased the development by the NIH Chemical Genomics Center on a titration-based, quantitative high-throughput screening platform (qHTS) to accelerate the identification of leads for drug discovery [13]. The platform was developed to screen compound libraries at seven or more concentrations (spanning nearly four orders of magnitude) in a 1536-well plate format to generate concentration response curves that would permit the derivation of SAR directly from the primary library screen. In addition, the approach also eliminated false positives and false negatives common to traditional single point HTS methods and can provide relative activities in highly focused libraries where single point HTS scores many compounds as active.

Using the qHTS platform, the NIH team screened a library of nearly 60,000 structurally diverse compounds in 7-15 concentrations with a fluorogenic substrate assay for wild-type GC activity. The assay was designed to pick up both inhibitors and activators of GC activity. The screen yielded 255 active compounds. Clustering and SAR analysis refined the active compounds into three classes of inhibitors chosen for further study—an aminoquinoline, a sulfonamide, and a triazine series. These series were expanded through commercially available analogs. Selected members of each class were shown to be inhibitors of GC with the most potent (an aminoquinoline) having a K_i of 21 nM. Fibroblasts homozygous for the N370S GC mutant showed an enhancement of GC activity upon compound treatment. The magnitude of enhancement was comparable to the previous Scripps iminosugar work although the NIH compounds showed improved selectivity against other sugar hydrolases. A significant increase in the lysosomal localization of N370S GC was convincingly demonstrated, suggestive of improved trafficking. The information-rich qHTS method appears to reduce the time required to sort out false positives, to increase the number of hits by reducing false negatives, and to permit a more rapid entry into an SAR analysis. How this plays out in speed to drug candidate remains to be evaluated.

A recent *Chemistry & Biology* article [14] from a Canadian consortium described the traditional HTS screening of 50,000 compounds for chemical chaperones (inhibitors) of β-hexosaminidase A (Hex), the defective lysosomal protein in Tay-Sachs and Sandhoff diseases. Three structurally distinct and competitive inhibitors were found with chaperone activity in patient fibroblasts. One can expect additional efforts to identify chaperone leads for other LSDs.

The identification of structurally diverse chemical chaperones with cell-based LSD activity is encouraging and opens the way for pharmacological optimization of lead compounds. The use of an inhibitor to enhance the activity of an enzyme is a somewhat dicey prospect in vivo. Clearly, the stimulation of properly formed enzyme by the inhibitor must be balanced against the direct inhibition of the enzyme. Not surprisingly, a bell-shaped dose response has been observed for the some of these chaperones reflecting the opposing functions of the small molecules but also suggesting that an effective dose can be achieved. The pulse-chase technique used in the cell-based chaperone assays, which stimulates of protein trafficking (pulse) followed by drug wash-out (chase) to reduce enzyme inhibition, does hint at a pulsatile dosing regime in patients. Although the NIH team screened for both inhibitors and activators, no activators were identified. Activators, if findable, would appear to have better therapeutic potential since the downside of enzyme inhibition would no longer be an issue. As more compounds are studied and new assays developed that more closely approximate defective intermediates in GC processing, it is possible that chemical chaperones that avoid active site binding may emerge.

Considerable pharmaceutical work lies ahead to attain effective drugs for LSDs. There is little doubt that the underlying biological problem and its possible chemical solution have reached a level of refinement that bodes well for the new treatments for these debilitating diseases.

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