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Sphingolipids in health and disease

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Our laboratory works on sphingolipids, important lipid components of eukaryotic cell membranes. Two major projects are currently underway. In the first, we are attempting to understand the roles that sphingolipids, particularly ceramide, play in signaling. In the second, we are attempting to delineate the molecular mechanisms by which sphingolipid accumulation in inherited metabolic disorders, such as Gaucher disease, Tay-Sachs (Sandhoff) and Niemann-Pick diseases, causes cell dysfunction, and hence disease.

In the first project, we have recently identified a gene family that regulates the synthesis of the important second messenger, ceramide. The original member of this family was the longevity assurance gene (*LAG1*)), shown to be required for ceramide synthesis in yeast. Gene database analysis subsequently revealed a new family of proteins containing the Lag1p motif in mammals. To date, we have demonstrated that three family members are involved in ceramide synthesis in animal cells, but remarkably, each one uses a different fatty acid substrate. Thus, over-expression of uog1 elevates synthesis of ceramides containing primarily stearic acid, over-expression of TRH1 elevates ceramides containing mainly stearic acid and arachidic acid, and TRH-4-overexpression elevates mainly palmitic acid-containing ceramides. We are currently attempting to determine if these proteins are *bone fide* ceramide synthases, or rather regulators of a putative endogenous ceramide synthase. In addition, we are studying the roles of each family member in ceramide-dependent signaling events.

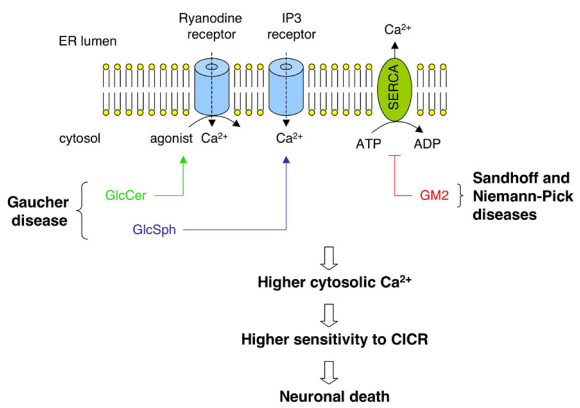

Fig. 1 *Neuronal calcium homeostasis is affected in Gaucher and Sandhoff diseases. In Gaucher disease, glucosylceramide (GlcCer) stimulates agonist-induced Ca^{2+}-release via the ryanodine receptor (RyaR) and glucosylsphingosine (GlcSph) acts as an agonist of the IP3 receptor. In Sandhoff and Niemann Pick diseases, ganglioside GM2 inhibits Ca^{2+}-uptake via SERCA. As a consequence, cytosolic Ca^{2+} is elevated in all three diseases which presumably leads to neuronal cell dysfunction and death.*

In the second project, we are studying the pathophysiological mechanisms of sphingolipid storage diseases, which are largely unknown. In a mouse model of Gaucher disease, in which glucosylceramide (GlcCer) accumulates, we demonstrated that there is a significant increase in the rate of Ca^{2+}-release from the endoplasmic reticulum (ER) via the ryanodine receptor (RyaR), resulting in elevated cytosolic Ca^{2+} levels which leads to enhanced sensitivity to agents that induce cell death. Microsomes prepared from human Gaucher type 2 and 3 brain samples show a similar elevation in Ca^{2+}-release (Fig. 1). Cytosolic Ca^{2+}-levels are elevated in a mouse model (the Hexb mouse) of Sandhoff disease (a form of Tay-Sachs disease), but in contrast to Gba neurons, this is caused by changes in the rate of Ca^{2+}-uptake into the ER via the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) rather than by changes in the rate of Ca^{2+}-release (Fig. 1). In addition, the rate of glycerolipid, particularly phosphatidylcholine (PC) synthesis, is

elevated in neurons cultured from Gba-/- mice due to direct activation of the rate limiting enzyme in PC synthesis, cytidylyltransferase (CCT), by GlcCer. A similar effect is seen in Gaucher macrophages, in which an increase in PC synthesis correlates with increased macrophage growth.

Finally, together with Israel Silman and Joel Sussman, we recently determined the X-ray structure of acid β-glucosidase (GlcCerase; Cerezyme), the enzyme used in enzyme replacement therapy in Gaucher disease (Fig. 2). The availability of this structure may now provide the possibility of engineering improved GlcCerase for enzyme replacement therapy, and of designing structure-based drugs aimed at restoring the activity of defective GlcCerase in Gaucher disease.

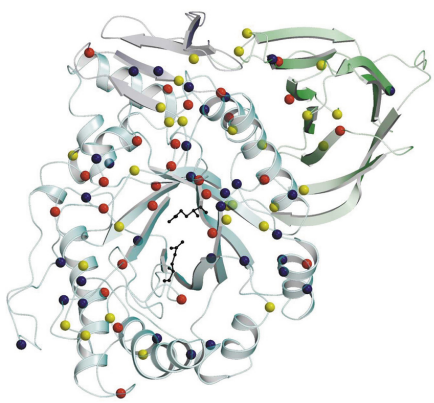

Fig. 2 *The 3D structure of glucocerebrosidase, the defective enzyme in Gaucher disease. Mutations causing severe disease are in red, mild disease in yellow, and those for which clinical data documenting disease severity are lacking, in blue. The active site glutamate residues are shown as balls and sticks.*

Selected Publications

Bodennec, J., Pelled, D., Riebeling, C., Trajkovic, S. and Futerman, A.H. (2002) Phosphatidylcholine synthesis is elevated in neuronal models of Gaucher disease due to direct activation of CTP:phosphocholine cytidylyltransferase by glucosylceramide. *FASEB J.*, 16, 1814-1816

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Lloyd-Evans, E., Pelled, D., Riebeling, C., Bodennec, J., de-Morgan, A., Waller, H., Schiffmann, R., and Futerman, A.H. (2003) Glucosylceramide and glucosylsphingosine modulate calcium mobilization from brain microsomes via different mechanisms. *J. Biol. Chem.*, 278, 23594-23599

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Riebeling, C., Allegood, J.C., Wang, E., Merrill, A.H. Jr., Futerman, A.H. (2003) Two mammalian longevity assurance gene (*LAG1*) family members, *trh1* and *trh4*, regulate dihydroceramide synthesis using different fatty acyl-CoA donors. *J. Biol. Chem.* 278, 43452-43459

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Buccoliero, R., Bodennec, J., van Echten-Deckert, G., Sandhoff, K., and Futerman, A.H. (2004) Phospholipid synthesis is decreased in neuronal tissues in a mouse model of Sandhoff disease. *J. Neurochem.*, in press

Futerman, A.H. and van Meer (2004) The cell biology of lysosomal storage diseases. *Nature Reviews Molecular Cell Biology,* in press

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