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Sphingolipids as regulators of neuronal growth, development and death

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Research into the function of sphingolipids (SLs) has undergone a renaissance in the past decade due to the realization that SLs are involved in a number of important regulatory processes in cell development. Over the past 3-4 years, our laboratory has focused on two major issues. The first concerns the regulation of SL metabolism during cell development, with a view to understanding the function of specific SLs at distinct stages of development, particularly neuronal development. The second seeks to understand why accumulation of SLs and glyco-SLs, such as occurs in the inherited metabolic disorders, Gaucher, Tay Sachs (Sandhoff), and Niemann-Pick A disease, results in cell dysfunction, and in some of the diseases, in the death of patients often due to neurological disturbances.

With respect to the regulation of SL metabolism during cell development, we have defined the pathways by which the sphingolipid second messenger, ceramide, is involved in the regulation of both neuronal development and also neuronal death. We have shown that exogenously-added ceramide can either stimulate neuronal development, or induce apoptotic cell death. Moreover, ceramide can be generated in vivo by neutral sphingomyelinase after binding of nerve growth factor (NGF) to the p75 neurotrophin receptor (in collaboration with Dr. Mike Fainzilber). Two down-stream players are involved in this pathway, namely jun-kinase and death associated protein-(DAP) kinase (in collaboration with Prof. Adi Kimchi). Finally, we are also studying whether ceramide generated from de novo synthesis is also involved in regulating cell death, and to this end are currently trying to identify a mammalian homologue of a putative yeast ceramide synthase.

Our work on sphingolipid storage diseases is becoming the central area of research interest in the laboratory. Despite years of study of the genetic and clinical basis of lysosomal sphingolipid storage diseases, no satisfactory molecular explanations exists to explain the pathophysiological mechanisms that cause the neurological symptoms and the early demise of patients suffering from some types of these diseases. Work from our laboratory has begun to provide clues that may provide molecular descriptions for the neuropathophysiology in the three diseases mentioned above. In a mouse model of Gaucher disease, there is a significant increase in the rate of calcium release from the endoplasmic reticulum (ER) upon stimulation of the ryanodine receptor, resulting in elevated cytosolic calcium levels (Fig. 1), which leads to enhanced sensitivity to agents that induce apoptotic cell death via the release of calcium from intracellular stores. The rate of glycerolipid, particularly phosphatidylcholine (PC) synthesis, is elevated in neurons cultured from glucocerebrosidase -/- (Gba-/-) mice, due to activation of the rate limiting enzyme in PC synthesis, cytidylyltranserase (CT) by glucosylceramide (GlcCer), the lipid whose levels are elevated in Gaucher disease. In addition, rates of axonal growth are faster in Gba knock-out neurons. In contrast, in a mouse model of Sandhoff disease (a variant of Tay Sachs disease), the Hexb knock-out mouse (Fig. 2), rates of axonal growth are slower, and rates of PC synthesis are also reduced. Cytosolic calcium levels are also elevated in Hexb neurons, but in contrast to Gba neurons, this is caused by changes in the rate of uptake of calcium into the ER rather than by changes in the rate of calcium release. As a consequence, Hexb neurons are more sensitive to thapsigargin-induced cell death, suggesting that elevated cytosolic calcium may contribute towards apoptotic cell death in Hexb neurons. Based on these data and studies underway using DNA microarrays, we are

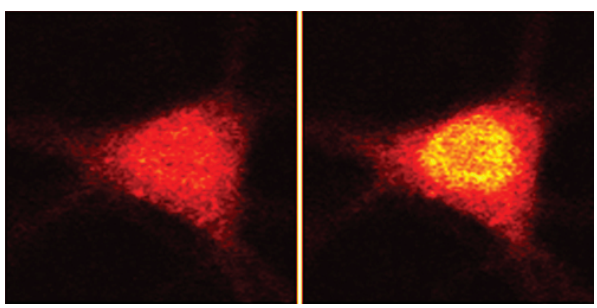

Fig. 1 *The left-hand panel shows levels of intracellular calcium, detected by calcium-sensitive dyes, after release of calcium from intracellular stores induced by caffeine. The right hand panel shows that levels of calcium release are much higher in neurons that have accumulated glucosylceramide, the lipid that accumulates in Gaucher disease. See Korkotian et al for more details. These studies were performed in collaboration with Prof. Menachem Segal and Dr. Ed Korkotian.*

beginning to obtain an integrated model that may explain the molecular basis of the neurological symptoms observed in this class of diseases.

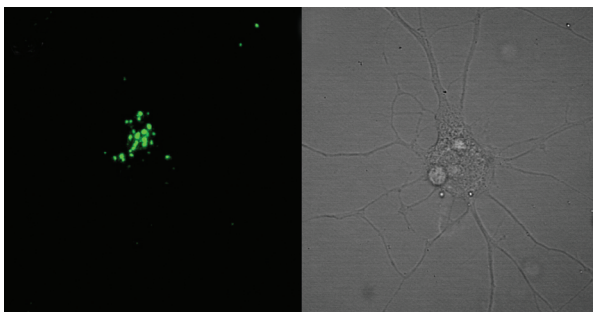

Fig. 2 *Neurons from a Hexb knock out mouse show elevated levels of ganglisoide GM2, which is localized to lysosomes. We are currently attempting to determine levels of extra-lysosomal GM2.*

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