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Lay summary

Neurobiology of Purkinje cells in NPC1.

We have analysed the storage lipids in the mouse model of NPC focusing on the part of the brain called the cerebellum, which is known to be affected in NPC disease. The aim was to determine over time how cerebellar lipid storage progresses to compare with the morphological studies of Purkinje neurons later in the project. Sphingosine (one of the lipids stored in NPC disease) was elevated as early as one week of age in the NPC1 mice where as glycosphingolipid levels rose later in disease, while cholesterol levels did not go up at all in this part of the brain.

It is also important to know where the lipids are accumulating. Staining of sphingomyelin and GM1 (two other lipids stored in NPC disease) was optimised and then used together with the cholesterol-label filipin to study Purkinje cells. Cholesterol accumulates in Purkinje cell bodies that die early in pathology, but also in a subset of Purkinje cells that survive until end stage disease. In agreement with another recent report (Elrick et al, Hum Mol Genet, 2010, 19(5):837-47), this indicates that while cholesterol storage is a very important marker of neuropathology, it is unlikely to be the key neuropathogenic factor in the cerebellum. Sphingomyelin and GM1 staining seems to be greater than normal near the axons of Purkinje cells, however further analysis is needed to confirm the precise location of the staining.

The use of cerebellar slice cultures from the nine day-old NPC1 mouse was tested, and preliminary evidence suggests that the defect in lysosomal calcium reported previously in NPC1 peripheral cells (Lloyd-Evans et al, Nat Med. 2008. 14(11):1247-55) is also present in neurons. The young age of the slices indicate that the calcium defect may well be an early event in NPC neuropathology.

Finally, a trial of healthy cerebellar progenitor cell transplantation was made in symptomatic 6-week old NPC1 mice to see whether the transplantation of new cells was viable and if the NPC1 cerebellum could support their development. The hope is that the transplanted cells are able to develop correctly into neurons and help replace the function of neurons lost in the disease. Transplanted cells survived and developed, many of them into mature Purkinje cells. This suggests that the NPC1-brain can accept new neurons through transplantation, however whether they can be delivered in high enough numbers or prove useful in delaying or reducing symptoms remains to be tested.

Update for September 2010

We have continued our analysis of the storage lipids in the mouse model of NPC. Previously we saw that cholesterol accumulation inside the cell bodies of Purkinje cells did not correlate with neurodegeneration, as the inclusions were found equally in Purkinje cells that die early in the disease, and those that are fairly resistant to the loss of NPC1 function. We have now have evidence that the same is true for other classes of lipids that also accumulate in this disease, GM1 and sphingomyelin, thus the accumulation of these lipids is unlikely to be the cause of neurodegeneration. When we previously looking at lipid levels in the whole mouse cerebellum across the time-course of NPC1, we noted a very early increase in the level of sphingosine, an early pathology noted in peripheral NPC1 cells. To see if there are differential sphingosine changes in disease-susceptible and disease-resistant areas of the cerebellum, we need a way of

analysing the different cerebellar areas independently. We have developed a way of surgically micro-dissecting the mouse cerebellum in such a manner that we can collect the different areas, and are now working on analysing whether there are differences in sphingosine levels in these different cerebellar regions.

The combination of an early rise in sphingosine levels and an early defect in lysosomal calcium regulation in the NPC1 cerebellum begged the question, “if these things are going wrong early in the development of the mouse NPC1 cerebellum, is there anything else going wrong?” We have noted a small but consistent number of Purkinje cells that seem to fail to develop properly. Normally, after these neurons are generated deep inside the brain, they move upwards until they reach the correct position. Instead, this small pocket of Purkinje cells seems to have gotten stuck halfway. While this is not the case for the majority of NPC1 Purkinje cells, it does indicate that their may be problems in neurons far earlier that previously thought, as the transition of these neurons to the correct position in the brain normally occurs before the mice are born. We are now looking at very young mouse cerebellar tissue to see whether this is the case, and look for other very early/developmental NPC1 phenotypes.

Finally, we have brought in a strain of mice in which all their cells express green fluorescent protein. These are the ideal mice from which to source easily monitored donor neurons for transplantation experiments without having to use less-reliable chemical labelling methods. As such, the planned work on cerebellar progenitor cell transplantation will be conducted as soon as possible.