

Neurobiology of Purkinje cells in NPC1

Dr. Ian Williams

Lay summary for March 2011

The death of Purkinje cells in the NPC1 cerebellum is thought to be a major cause of the problems concerning motor coordination in this disease. However, it seems that not all Purkinje cells are affected in the same way in this disease and can be looked at as three distinct groups, one group that dies off fairly early in disease, a second group that dies off significantly later in disease, and a third group that, over the lifespan of the NPC1 disease mouse are resistant and survive (see the examples below). We have taken advantage of these three groups of Purkinje cells, as the differences between them will give us insights into what aspects of the disease process are killing these important neurons, and what is different about the Purkinje cells which survive. This information will be key to inform future therapeutic approaches aimed at maximising Purkinje cell survival.

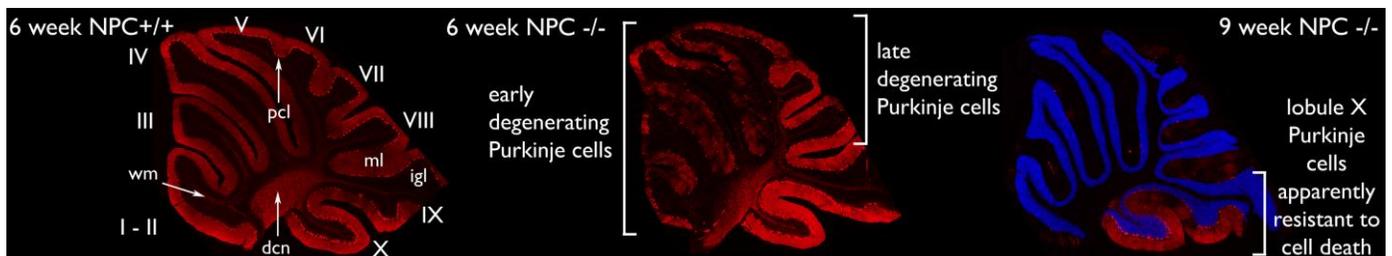
We have been looking at the differences in lipid storage, inflammation, and protein expression in these three different groups of Purkinje cells. So far, there have been no differences in the storage of cholesterol or GM1 ganglioside in the three different groups, and are now looking at another lipid that appears to be the first lipid to be stored in NPC1 cells, sphingosine.

There are very striking differences in brain inflammation between these different groups of Purkinje cells, with higher levels of damaging inflammation in the areas which die sooner in disease, and some evidence of a positive/protective response in the areas which survive the longest. This tells us that the different groups of Purkinje cells are not just dying off at different times but are interacting with the brain immune system differently (see examples below).

At the last NNPDF conference, there was a presentation that described the use of Calbindin as a potential biomarker in NPC1. Calbindin is a protein naturally expressed at high levels in Purkinje cells. According to our work so far, calbindin levels in NPC1 Purkinje cells are initially lower than normal. The levels of Calbindin then seem to recover dramatically, and even exceed what is found in normal Purkinje cells. Higher levels of calbindin correlate with the neurons that survive the longest, indicating that calbindin may not only be important as a biomarker, but also have a role to play in the disease itself.

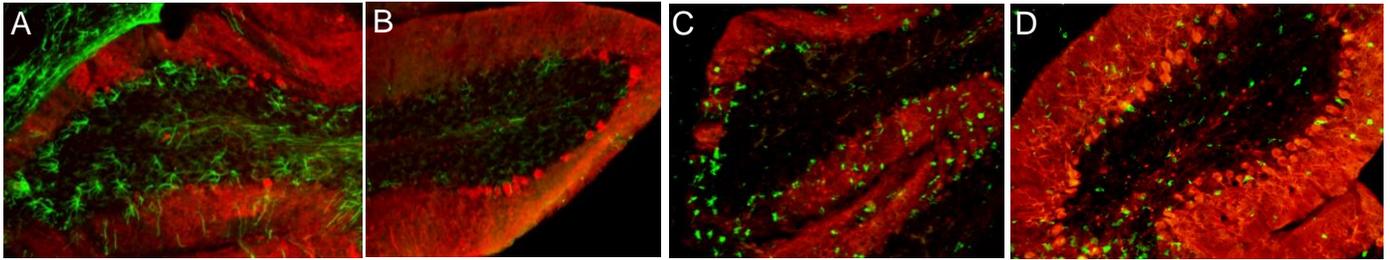
Finally, studies done by colleagues in our laboratory have shown that heterozygous NPC1 mice show signs of typical NPC1 disease much later in life. We are now studying the brain tissue of these mice to see if there are signs of neuropathology. If there are, the heterozygous NPC mouse could provide a very useful mouse model of late-onset NPC1 and greatly aid our understanding of disease mechanisms.

Patterned Purkinje cell loss in the NPC1 cerebellum



The cerebellum is the part of the brain mainly concerned with the coordination of motor control. The key neurons involved in this are the Purkinje cells, in these images they are the red cells which form a continuous layer on the outer edge of the cerebellum, which itself is split anatomically into ten lobules (as labelled in the first picture). The middle picture is of a 6-week old NPC1 mouse cerebellum, and you can see the Purkinje cells have begun to die, seen as gaps in the continuous red layer. It is quite striking that the cell loss is not even, and that some cells die long before others. By 9-weeks old (far right), most of the Purkinje cells are lost with the exception of the ones in lobule X. Understanding why some of these cells are affected so early and why others seem so resilient is important in figuring out the basis of neurodegeneration in NPC1 disease.

Patterned inflammation in the NPC1 cerebellum



The brain is separated from the regular immune system by the blood brain barrier. Instead, the brain has specific cells to fulfil the role of immune system, called astroglia and microglia. They exist in the brain in a dormant state, and are activated in response to disease or injury. In images A and B, you can see astroglia coloured green against the background of Purkinje cells, coloured red. Image A is from lobule III, part of the "early degenerating area", and is full of astroglia, whereas image B is from lobule X the "non-degenerating" area, where there are very few astroglia. In images C and D, microglia are coloured in green and the same pattern arises, with lobule III full of large rounded active microglia, while the microglia in lobule X are generally small and inactive. This shows the disease is affecting the two cerebellar regions very differently, and understanding why this is the case will be important in discovering how best to slow or avoid neurodegeneration in NPC1 disease.