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TITLE: Establishment of Central Nervous System Cell Lines from NPC-1 Disease Mice for Pathogenic and Therapeutic Studies

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PROJECT DESCRIPTION

The goal of this study is to establish immortalized cell lines of the major cell types in the central nervous system (CNS) from Niemann-Pick disease type C1 (NPC1) mice. These mice carry a mutation in the NPC1 gene which has been well characterized and shown to represent a bonafide model for the human disease. NPC1 is a hereditary lysosomal storage disease (LSD) that results in the intracellular accumulation of unesterified cholesterol and specific gangliosides. Unlike most storage disorders, the genetic mutation does not involve a lysosomal enzyme but rather a membrane protein that is thought to be involved in the subcellular "retroendocytic trafficking" of cholesterol and possibly gangliosides. At present no CNS cell type lines of NPC1 exist, despite the fact that CNS pathology represents a dominant feature of this disease. Such lines would be an extremely useful research tool as the subcellular function of the native protein and the resulting defect is still poorly understood, requiring further study at the cellular level. Furthermore, there are significant inherent differences between the various CNS cell types and other cell types, both in terms of subcellular vesicular pathways and in the metabolites involved. Thus, research on the cell biology of NPC1, which has largely been carried out on fibroblast-like cells, provides a poor model for understanding what is going on in neurons. This in turn limits the ability to design rational strategies for treating the CNS. Appropriate neural cell lines would also be invaluable in preliminary evaluation of such strategies prior to difficult in vivo testing which poses the additional challenge of effective delivery methods to access the CNS.

In the course of our previous studies on pathogenesis and therapy employing CNS cell cultures from NPC1 mice, we discovered a spontaneous cell transformant showing a high and sustained proliferation rate. We now wish to further characterize the nature of this cell line. Furthermore, we propose to develop cell lines of astrocytes, oligodendrocytes, microglia and neurons which closely reflect the original properties of the natural cell population yet can also be easily induced to robustly proliferate to generate large numbers for study. To achieve our goals, we will employ a strategy we have had previous success with. The cells of interest will be directly isolated from unique transgenic mice that carry both the BALB/c npc1nih mutant gene and a double -conditional immortalizing gene. Using these mice and standard methods of cell culture, we will obtain the desired cell types which will already express both a recognized NPC1 mutation and a gene that allows us to turn on and off the ability to proliferate. This strategy circumvents many problems associated with the more common approach of introducing genes into cells in culture which can lead to cell lines that poorly represent the natural cell type because of dedifferentia tion and clonality. Once cell lines are obtained, the rate of proliferation will be determined, and their retention of expected phenotypic properties validated. We will employ appropriate growth

conditions to support development of mature characteristics of each of the cell types and compare this to cells in primary cultures from the original NPC1 model mice. Similarly, using established flourescent markers and immunofluorescent techniques together with confocal microscopy and quantitative image analysis, we will evaluate lysosomal storage of unesterified cholesterol and accumulation of ganglioside.

Once established and characterized, the results will be reported and the cell lines made readily accessible to other researchers investigating NPC disease. These lines will prove extremely useful to our own continuing studies on NPC and provide an important tool for accelerating the identification of an effective CNS therapy.

Final STATUS REPORT
Dated 1/29/2008

NPC1 disease is an inherited disorder that severely affects the central nervous system (CNS) and for which there is no cure. The disease involves a defect in a transmembrane protein that is thought to participate in the "retroendocytic" trafficking of cholesterol and in the trafficking and/or steady state of specific glycolipids including GM2 ganglioside (GM2) and GM3 ganglioside (GM3). There is still a limited understanding of the subcellular function of the NPC1 protein and most investigations on this have used non-nervous system cells. Yet cells of the CNS are highly specialized and the precise pathogenetic mechanisms that lead to neural dysfunction here are unknown. Such studies as well as the rational development of therapeutic strategies would be greatly facilitated by the availability of lines of CNS cells that express a suitable NPC1 mutation and a mature cellular phenotype, but no lines with these properties have been available.
In this project we have characterized a spontaneous multipotent neural cell line arising in brain cell cultures from the BALB/c npcnih mouse, an accepted model for NPC1 disease. This line, named NPC-ST1, is capable of generating the three major neural cells of the CNS: neurons, astrocytes and oligodendrocytes. We have optimized culture protocols for this line that individually favor the survival and differentiation of each of the specific cell types for suitable study of mature as well as developing cells. The line also displays appropriate accumulation of unesterified cholesterol, a hallmark of NPC1 disease. Some cells in the line exhibit the presence of GM2 ganglioside which is known to accumulate within sub-populations of cells within the diseased CNS.
We have also produced "conditionally immortalized" cell lines from unique hybrid transgenic mice we generated. These carry intrinsic genes that allow one to alternatively stimulate unlimited cell division to permit the generation of large numbers of cells for study, and when desired, to block this growth and return cells to a more normal state allowing progression into a mature cell stage. We have established conditionally immortalized microglia and astroglial cell lines carrying the mouse mutation. In addition, in parallel we established immortalized lines of the same types that express the native NPC1 protein to serve as normal controls for experimental studies. Both astroglial lines show expression of the astrocytic marker, GFAP, and appropriate cell-specific differentiation. Interestingly, in addition to displaying cholesterol accumulation, the disease line shows some phenotypic differences related to differentiation and reactive astrocytosis as compared to the normal line. Furthermore, initial observations suggest cholesterol accumulation substantially varies within the astrocyte population, surprisingly with lower

amounts found in more differentiated cells. These findings may lead to new insights on the role and impact of NPC1 deficient astrocytes in the disease process. Microglia are the macrophages of the CNS and have been implicated as important players in NPC1 disease progression. The disease microglial cell line shows cholesterol accumulation that is strikingly greater than found in the astrocyte line. This is compatible with some prior observations reported for NPC1 disease mouse brain. Both the disease and normal NPC1 microglial lines were confirmed positive for three microglial specific markers. Of these, one antibody for Iba-1, which is an important calcium binding protein enriched in microglia, shows less intense staining in the disease line than in the normal line, and may point to unanticipated changes occurring within the diseased microglial population. Neither the astrocyte or microglial disease lines show GM2 accumulation, but trace amounts of GM3 can be detected in the microglial line. Finally, we have also initiated efforts to generate a similar set of immortalized lines of oligodendroglia, the cells responsible for myelination important to nerve cell conduction within the CNS. We expect all these unique cell lines will prove valuable to ongoing and future investigations of NPC1 pathogenesis and therapy.

PUBLICATIONS:

No Publications on this Work To Date