NNPDF-Funded Research Grant # 30

TITLE: Functional Analysis of NPC1
PROJECT INVESTIGATOR: Anita H. Corbett, Ph.D.

PERIOD: 8/1/2002 - 7/31/2003

PROJECT DESCRIPTION

Niemann-Pick Type C Disease is a devastating genetic abnormality which debilitates otherwise healthy children and leads to an early death and for which there is, currently, no treatment. Recently the specific genes at fault were isolated but the function of these genes has not been clearly identified. The simple organism, yeast, has been used extensively to understand how different genes work within cells. In fact, the 2001 Nobel Prize for Physiology or Medicine was won by three researchers who used yeast to study the genes that control how all cells grow. Their research led to the identification of many of the genes that control normal cell growth and to the realization that many of these genes are defective in patients who develop cancer. This led to medical advances in the treatment and diagnosis of many human cancers. We hope to now apply the yeast model system to understand the problems that occur in patients who are afflicted with Niemann-Pick C (NP-C) disease. The very simple yeast cells we study in our laboratory are similar to the cells that make up the human body. Since processes that occur in the yeast cell are similar or identical to those that occur in the cells in our bodies, many laboratories use yeast cells to study how human cells work. There are many advantages to working with yeast cells, not the least of which is that yeast cells grow very quickly so experiments can be accomplished very rapidly. This means that information that would take years to learn in other systems can be gathered in months or weeks using yeast. Additionally, yeast cells are very inexpensive to grow so a relatively small amount of money can lead to important scientific discoveries and the development of important tools for medical diagnosis and treatment. For these reasons, many biomedical and pharmaceutical companies use yeast in initial screens to identify new drugs for the treatment of human diseases. We hope to exploit some of these advantages to make rapid scientific advances that could lead to an improved quality of life for patients with NP-C disease. Yeast cells have genes that are similar to the two genes that have been found to be defective in patients with NP-C disease, NPC1 and NPC2/He1. This suggests that yeast can be used to study the changes that occur in cells when these genes do not work properly. There are two basic goals of our proposal. The first is to test whether these genes work in the same way in yeast as they do in humans. The second is to test whether we can use yeast cells where we have replaced the yeast NPC1 gene with a defective NPC1 gene from a patient to study the function of that patient's defective NPC1 gene. We think that both sets of experiments could provide important tools that could be useful in the diagnosis and treatment of NP-C disease. The preliminary data in our proposal show that yeast NPC1 is found in the same place within yeast cells as it is in human cells. This suggests that the yeast NPC1 protein may function in the same way that the human protein functions. We have proposed experiments to directly test this idea. If we find similarities, this will suggest that the very simple yeast cells could be an important tool for understanding the basis for the problems that occur in patient cells. Our other preliminary data show that we have identified a change (called a

phenotype) in yeast cells that do not have a working NPC1 gene. To our knowledge this is the first phenotype that has been observed in yeast cells that have a defective NPC1 gene. We can also demonstrate that giving these yeast cells back a working copy of the yeast NPC1 gene fixes this phenotype. This gives us a tool to test whether NPC1 genes from patients are able to work properly within cells. We will use this tool to test whether changes within patient NPC1 genes destroy the function of those genes. This would also be a way to test whether there is any defect in the NPC1 gene before a patient shows any clinical symptoms of NP-C, which could allow doctors to confirm or rule out the possibility that a patient will develop NP-C in the future. We hope that these very initial experiments will open a new field for studying the function of both the NPC1 and NPC2/He1 genes. It is important to understand what these genes do in normal cells before we can understand what goes wrong in NP-C patient cells. This proposal will test whether a very simple yeast based system can be a useful tool to answer some of the most important questions about the cause of the disease, which could lead to accurate diagnosis and effective treatment.

FINAL STATUS REPORT
Dated 7/31/2003

Our work attempts to use a very simple single-celled organism, the budding yeast Saccharomyces cerevisiae, to understand what goes wrong in the cells of patients with Niemann Pick Type C disease. This type of yeast is that same yeast that people use when they bake bread or brew beer. It is very easy to work with and it grows very fast so we can do experiments quickly and hopefully learn things that will help NP-C patients as quickly as possible. Despite the fact that there are some obvious differences between humans and the single-celled yeast, our studies show that yeast have the proteins that are defective in patients that have NP-C disease, NPC1 and NPC2. We have been studying the yeast proteins to convince ourselves and others that these proteins do the same thing in yeast cells that they do in human cells. Our studies do strongly argue that the proteins in yeast probably work in much the same way as they do in humans.

However, one problem that researchers have always had in correlating yeast to humans for NPC research is that yeast do not perform complex functions like walking, talking or reasoning. So how do we know when a yeast is "sick with NPC"? Until now, we have had no way to see that a "yeast with NPC" was sick because no one had identified any symptoms. NPC yeast appeared to act just like healthy yeast. But our recent experiments have revealed a measurable difference between yeast with NPC and healthy yeast; NPC yeast are resistant to the ether lipid drug edelfosine while healthy yeast are not.

This finding is significant because it means that we can use the yeast as a "model system" to study the function of the NPC1 and NPC2 proteins and to test various compounds on NPC yeast to see what might restore them to normal. Results of these tests will suggest compounds to test as therapies in NPC animal models. Learning more about the function of the NPC1 and NPC2 proteins should help us understand what goes wrong in patients where these proteins do not work properly and should also give us ideas about ways to correct this deficiency.

PUBLICATIONS:

http://www3.interscience.wiley.com/cgi-bin/fulltext/118668899/PDFSTART

http://ec.asm.org/cgi/reprint/4/11/1851