Can we do stem cell experiments to alleviate manifestations of PXE?

Stem cells have the unique ability to differentiate into new cell types. This creates possibilities to repair diseased, dysfunctional, or injured tissues. Little research has been completed with stem cells to alleviate manifestation of PXE in humans due to the ethical issues and the extreme expense of this type of research. One experiment used stem cell therapy on mice that showed signs of PXE. This study concluded, "stem cell research in animal models has many more questions to answer and a long way to go before these and other techniques may be used as therapies for humans." Below is a detailed summary of the study.

Stem Cell Therapy Experiments in the PXE Mouse

Lay summary by Rachel Koren, Research Assistant, PXE International, and Christine Vocke, Director of Education and Support, PXE International
This lay summary was first published in the February 2013 eNewsletter

> Jiang Q, Takahagi S, Uitto J. Administration of bone marrow derived mesenchymal stem cells into the liver: Potential to rescue pseudoxanthoma elasticum in a mouse model (Abcc6(-/-)). *J Biomed Biotechnol*. 2012 Nov. [Epub ahead of print] Article here

In the search for a treatment for pseudoxanthoma elasticum (PXE), researchers have begun exploring stem cells as a possibility. Stem cells are cells within the body from which all other cells with specialized functions are derived. Previous studies have shown that stem cell transplants can be used to replace damaged cells and restore bodily functions that were lost by the damaged cells. Much anticipation has surrounded research on stem cell transplants because of their potential to produce a cure for currently incurable diseases.

PXE has been confirmed as a metabolic disorder caused by a protein that malfunctions in the liver. Stem cells derived from bone marrow, called mesenchymal stem cells (MSCs), are of particular interest to PXE researchers because previous studies have shown their capacity to develop into liver cells and be successfully used in patients with liver disorders. This study, conducted by researchers in Dr. Jouni Uitto's laboratory at Jefferson Medical College, explored the effects of several techniques for introducing MSCs into PXE knockout mice.

PXE knockout (KO) mice are bred to have no functioning copies of the *Abcc6* gene so that they develop signs similar to PXE in humans, including mineralization of elastic tissues in the skin, eyes and arterial blood vessels. For this study, PXE KO mice were additionally bred to be immunodeficient, meaning that their immune systems did not work properly. When receiving a transplant of any kind, unless from an identical twin, the recipient's immune system must be suppressed in order for the body to accept the foreign transplant. If immunosuppressants are not used, the immune system may fight against the stem cells that are transplanted into the

body, and the transplant will be unsuccessful. Thus the mice for this study were bred with both PXE and immunodeficiency so that they would be able to accept the stem cell transplants.

At the age of four weeks, the mice were anesthetized and underwent a partial hepatectomy (HPx), a removal of half of their liver. This surgery promoted the production of stromal cell derived factor (SDF-1). SDF-1 is a protein produced throughout the body, and especially where an organ or tissue is injured. SDF-1 is responsible for a process called chemotaxis. Chemotaxis is the movement of cells in response to a chemical stimulus. One day after the surgery, MSCs were transplanted into the PXE KO mice by injection into the spleen. When stem cells are transplanted, they take the place of cells that have been lost or damaged. The HPx procedure allowed the researchers to see how the stem cells could be best prompted to migrate from the spleen to the liver and rebuild the damaged liver.

Two types of stem cells were used to evaluate two activities: 1) how effectively the stem cells differentiated, or developed into liver cells, and 2) how quickly they moved to the liver after being injected into the spleen. Both sets of stem cells were derived from the bone marrow of mice without PXE and then purified. Half of these purified stem cells were additionally modified to overproduce a protein called a CXC chemokine receptor (Cxcr4). Cxcr4 works specifically with SDF-1, the protein produced when the PXE KO mice received the liver surgery. Cxcr4 and SDF-1 work like magnets, attracting each other specifically to promote proper cell migration. Researchers hypothesized that an overproduction of Cxcr4 would promote faster migration to an area that was producing a lot of SDF-1, and used the unmodified MSCs as a comparison.

Researchers studied the responses of both sets of stem cells in vitro (outside the body) as well as in vivo (inside the body) in the mice after they were injected into the spleen. The cells studied outside the body were easy to observe. The cells studied inside the mice were tagged with a fluorescent dye so that they could be seen by an imaging system.

Stem cells were shown to properly develop into liver cells in vitro when cultured in a growth medium designed to induce the cells to develop into liver cells. The cells studied in vitro were also found to be capable of expressing the *Abcc6* gene. This is particularly encouraging because the loss of this gene, and especially the loss of its expression in the liver, appears to be the cause of PXE symptoms. The ability of these new cells to express the gene, even when the original cells of its host do not have functional copies of the *Abcc6* gene, shows a potential for these stem cells as a therapy for PXE.

In examining the migration of normal MSCs versus the stem cells that were modified to over-produce Cxcr4, the researchers found their hypothesis to be correct. In the cells that were studied in vitro, the Cxcr4 stem cells were found to migrate in greater number and with greater speed toward an area rich with SDF-1. In vivo, the results were the same. The cells with enhanced Cxcr4 production migrated from the spleen to the liver more quickly and in greater numbers than the MSCs that had not been modified.

The use of stem cells as a therapy for genetic disorders is an exciting possibility, though the research is far from conclusive. Researchers continue to work to find the best techniques and methods for possible use in therapies. However, stem cell research in animal models has many more questions to answer and a long way to go before these and other techniques may be used as therapies for humans.