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Project Title: Therapeutic Potential of Human-Induced Pluripotent Stem Cells (IPSCs) in the Sandhoff Disease Mouse Model of Lysosomal Storage Disorders

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Lysosomal storage diseases (LSDs) are due to defects in enzymes that normally degrade fats and other cellular substances that are no longer useful. Not only are cells unable to get rid of this waste but also the toxic byproducts that build up can kill nerve cells, leading to harmful symptoms. The symptoms reflect what organ is directly affected, be it bone marrow (effecting blood production), liver, or spleen. Sometimes the affected region comprises the brain and spinal cord. For most affected organs, treatment includes replacing the missing enzyme or gene (in other words, gene therapy) or performing a bone marrow transplant to replace the defective cells. Afflicted organs not helped by these therapies include the brain and spinal cord due to the blood brain barrier, which acts like a brick wall to block therapeutic substances injected into the bloodstream from accessing the brain. Hence, nerve cells die, resulting in devastating symptoms such as those involved with movement, speech, memory, thinking, and personality.

In our work, we hypothesized that if we transplanted cells directly into the brain, we could circumvent the blood brain barrier and deliver active proteins to damaged nerve cells to compensate for the defective proteins, and we would have a good chance to defeat these diseases. Stem cells are one possible way to deliver the genetic material that encodes these normal proteins. The most plentiful stem cell source is embryonic tissue. Embryonic stem cells (ESCs) are pluripotent (Latin for "many + having power"), meaning they can become nearly any cell type. Since ESCs also present many ethical issues, we have been seeking alternative stem cell sources.

Fortunately, stem cells can be had from other sources. While stem cells from alternative sources avoid the ethical consequences of embryonic tissue, they have other limitations in that most cells of a defined type, such as bone, skin, or nerves, have changes made to their genetic material that prevent them from becoming other cell types. Fortunately, we can treat cells with certain protein factors that "reprogram" them, restore their pluripotency or make them act young again. To do this, we used methods previously developed to reprogram human skin cells to make them pluripotent. We call these cells "human induced pluripotent stem cells", or hiPSCs. We then treated the hiPSCs to transform them into young nerve cells called "neural stem cells" (NSCs) because NSCs can be successfully transplanted into the brain. Our engineered cells are referred to as iPS-NSCs.

Do these induced cells look and behave like cells from embryonic tissue? We have determined that they do have typical nerve cell features and, indeed, express nerve-specific proteins. We next observed the behavior of our iPS-NSCs in a mouse that models Sandhoff disease (SD), an LSD cousin of Tay-Sachs disease. This animal model has been genetically altered to produce neurodegenerative symptoms that mimic those of LSDs, such as difficulty in walking, reduced muscle strength, and shortened life span. If we hope to delay or defeat these symptom onsets, our stem cells must be able to deliver healthy proteins to the affected areas. However, when we injected these iPS-NSCs into the mouse brains, they instead clustered close to the injection site, which essentially prevented them from

delivering their cache of functioning proteins to the affected nerves cells and replacing missing cells. So we then considered what to do to enhance migration of these stem cells.

Earlier, we discovered that a protein, a "chemokine", that participates in inflammation, can also promote migration of NSCs to damaged areas. If we could activate a certain chemokine receptor on iPS-NSCs (of course, without triggering the bad inflammation that chemokines can do), it might stimulate iPS-NSCs to home in on exactly the locations where they are needed. Thus, we synthesized just such an artificial protein (called a peptide), which acts like a drug that activates the receptor. Now when iPS-NSCs were transplanted to SD mouse brains that had received this very simple and safe new chemokine drug, iPS-NSC migration improved, and the cells could travel to areas of the brain in need.

For our future plans, we will seek other ways to enhance the potential of iPS-NSCs by increasing their ability to migrate using this drug. We will engineer iPS-NSCs to carry normal copies of the hexaminidase protein otherwise missing in SD and Tay-Sachs patients (this approach should also work for proteins missing in other LSDs). We will then test whether transplanting these engineered cells in SD mice improves their muscle strength and lengthens their life span. Based on present successes, we are confident our findings will eventually translate into effective stem cell-based therapies for LSDs.