

2008 Research Grants

Over \$3 million awarded for research grants since 2000!

The National MPS Society has awarded \$499,000 for research grants in 2008. The funding that the Society provides has been and continues to be crucial as we move forward with our mission to find the cures.

Drs. Bigger, Montano, Sands, Serafini, and Steet were awarded the general research grants of \$60,000 each. Drs. Brunetti-Pierri and Crawford were each awarded \$65,000 for MPS II research, and Dr. Ballabio was awarded the MPS III grant for \$60,000. Each grant is for two years, and the researchers will receive half of the total each year.

We received 22 letters of intent from researchers throughout the world for the eight research grants offered in 2008. After reviewing those letters, our Scientific Advisory Board review committee requested full grants proposals from 12 researchers.

This year we collaborated with two foundations to offer an MPS III partnership grant. We did not have enough funds in the MPS III research category to fund a grant, and we are very grateful to the Childrens Medical Research Foundation and Bens Dream Foundation for helping to fund this grant. Opportunities such as this ensure that our research dollars are not dormant for a year as we await additional donations to fund a grant. Its also a wonderful opportunity for the MPS community to join together as we strive to meet our common goal to find the cures.

Money from our research funds supported the expert Newborn Screening meeting held February 1, 2008 and reported in summer 2008 Courage. For the last three years the UK MPS Society has funded the research of Prof. Grzegorz Wegrzyn at the University of Gdansk in Poland, Development of gene expression-targeted isoflavone therapy for MPS III.” The UK requested support this year from our sister organizations in the International MPS Network to fund the extension 4th year. The National MPS Society has funded the requested \$4,000 for Prof. Wegrzyns research.

Dr. Nicola Brunetti-Pierri
Baylor College of Medicine, Houston, TX
HDAd gene therapy for lysosomal storage disorders”

Lysosomal storage disorders (LSD) often present with severe neurologic involvement. However, currently available treatments are not effective to treat this significant problem. Both enzyme replacement therapy and gene therapy have failed to show a significant neurologic improvement because the deficient enzyme is transported from the bloodstream to the brain with very low efficiency. To overcome this obstacle, we propose to inject a gene therapy vector directly into the brain fluid (called cerebrospinal fluid or CSF) through a simple and minimally invasive lumbar puncture. By this method, our gene therapy vector will transfer the gene encoding for the deficient lysosomal enzyme to the brain cells lining the CSF spaces. We hypothesize that these cells will secrete the enzyme in the CSF and through the CSF circulation it will diffusely penetrate into the brain to correct the storage disease. The goals of this proposal are to test the efficacy of this approach in mice affected with MPSII and the safety in nonhuman primates (baboons) because large animal models can better predict the outcomes in humans. Therefore, the studies included in this proposal have the potential to generate clinically relevant results which could be applicable for all LSD with neurologic involvement.

Dr. Brett E. Crawford

Zacharon Pharmaceuticals Inc., La Jolla CA,

Glycosaminoglycan inhibitors as substrate reduction therapies for MPS II”

Mucopolysaccharidosis (MPS) is a collection of genetic disorders caused by mutations in genes encoding enzymes required to degrade carbohydrate structures known as glycosaminoglycans (GAGs). The impaired degradation caused by these mutations leads to accumulation of GAGs within cells which in turn leads to serious multi-system disease. Iduronate sulfatase is a critical component of the GAG degradation system, this enzyme is responsible for removing the 2-O-sulfate residues in GAGs that are being degraded. In MPS II patients, the impaired function of the iduronate sulfatase leads to the accumulation of GAG fragments with 2-O-sulfate groups which cannot be degraded. We have discovered compounds that inhibit GAG 2-O-sulfation. These compounds are potentially the starting point for a novel substrate reduction therapy for MPS II. Because these compounds can reduce the amount of 2-O-sulfated GAGs made by cells, it is possible that they could reduce GAG accumulation due to an impaired iduronate sulfatase. In this application, we propose to test these compounds in MPS II models to determine if inhibiting GAG synthesis can reduce GAG accumulation and alleviate symptoms of the disease.

Dr. Andrea Ballabio

TIGEM (Telethon Institute of Genetics & Medicine), Naples, Italy

Modulation of autophagy as a potential therapeutic approach for MPS”

Autophagy is a lysosome-mediated degradation pathway in which large portion of cytosol are sequestered in specific vesicles (autophagosomes) and then degraded upon fusion with lysosomes. We demonstrated in two different mouse models of mucopolysaccharidosis, the Multiple Sulfatase Deficiency (MSD) and mucopolysaccharidosis type-III A (MPS-III A), an impairment of autophagy caused by inefficient fusion between autophagosomes and lysosomes. This results in an abnormal accumulation of different toxic substrates that ultimately lead to cell damage and death. Our results are supported by independent studies demonstrating that a dysfunction of autophagy also occurs in other forms of lysosomal storage diseases. The goal of this project is to exploit novel therapeutic strategies to treat MPS pathology based on the prevention/removal of the toxic substrates that accumulate as a consequence of inefficient autophagic degradation. This will be achieved using both pharmacological and genetic approaches. Our results will be instrumental to develop new therapeutic strategies in human patients.

Dr. Brian Bigger

Royal Manchester Children’s Hospital, Manchester, UK

The effect of heparan sulphate on stem cell homing and engraftment in MPS I”

MPS I Hurler is a fatal genetic disease caused by the lack of a specific enzyme which helps to break down large sugars called glycosaminoglycans (GAGs) in the body. The only treatment is stem cell transplantation, where cells from healthy donors replace patients own bone marrow and produce the missing enzyme. Only around half (56%) of these transplants are successful first time; often a second or third transplant is needed and this can be more risky. In Hurler patients, two types of GAG, heparan sulphate and dermatan sulphate, cannot be broken down, and instead they accumulate inside as well as on the outside of cells. Heparan sulphate helps cells in the body to signal to each other, and is needed by stem cells to home to the bone marrow following transplantation. It is also one of the components of extracellular matrix (ECM); the material that fills the spaces between cells. We have shown that normal cells from a stem cell transplant home differently across ECM from MPS I mice and want to identify if this or other factors cause stem cell transplants to fail. This will help us develop safer transplants for patients with MPS I Hurler.

Dr. Adriana M Montano

Saint Louis University School of Medicine, St Louis, MO

Identification of genes for keratin sulfate biosynthesis: toward development of RNAi mediated therapy”

The main goal of this research is to establish a novel therapeutic system for MPS IVA (Morquio A) by reducing the synthesis of the accumulated substrate (keratan sulfate) in the skeletal tissue to improve the bone lesions. In this proposal, we will test a new approach by partially blocking the synthesis of skeletal keratan sulfate mainly produced in cartilage cells of Morquio A patients. First, two candidate genes responsible for the synthesis of skeletal keratin sulfate will be characterized functionally. Afterwards the enzyme(s) responsible for the synthesis of skeletal keratin sulfate will be attenuated by a recently developed RNA interference method. The targeted gene is suppressed at the RNA level. Our modified RNA interference system will be unique and novel since the therapeutic agent is targeted to the major bone matrix, hydroxyapatite, by attaching a short acidic peptide to the agent. The attenuation of synthesis of the enzyme(s) will be tested initially in vitro in cartilage cells of MPS IVA patients. Successive preclinical trial on animal model(s) will provide critical information leading to human clinical trials. This new approach could be applicable to all types of MPS which store different types of glycosaminoglycans and suffer from bone lesions.

Dr. Mark S. Sands

Washington University School of Medicine, St. Louis, MO

Metabolic adaptations and phenotypic consequences of blocking lysosomal recycling”

Lysosomal storage diseases (LSDs) are caused by a deficiency of enzymes responsible for recycling material in cells. This recycling serves an important purpose by saving the cell energy. In LSDs recycling is interrupted and storage material builds up in lysosomes, likely contributing to disease. The amount of energy saved by a normal cell by lysosomal recycling is equivalent to the amount of inaccessible energy (storage material) in the lysosome of an affected cell. This can be an enormous amount of energy over the life of a cell. Since lysosomal recycling is blocked in LSDs, an affected cell has to expend more energy to carry out its normal functions in order to make up for the inaccessible energy stored in the lysosome. In an organism, this will result in a deficiency in fat stores. We previously documented this effect in five different mouse models of lysosomal storage disease. We are currently conducting experiments in mice to ascertain what adaptations the affected cells are making to the lack of recycling and how this contributes to the symptoms and progression of the disease. The knowledge gained from these studies will be used to design and test the effectiveness of dietary interventions.

Dr. Marta Serafini and Dr. Ettore Biagi

Dulbecco Telethon Institute at M. Tettamanti Research Center Clinica Pediatrica Univ., Monza, Italy

Marrow mesenchymal stem cell therapy for MPS I”

Affecting one in 100,000 children, Hurler syndrome is a rare genetic disorder where the IDUA enzyme, which normally breaks down the mucopolysaccharides dermatan and heparan sulphate, is missing. These mucopolysaccharides build up in all tissues in the body causing progressive deterioration and abnormal function of multiple organs. Hematopoietic cell transplantation (HCT) is one of the most promising treatments available that retards the progression of the disease. The clinical success of HCT as therapeutic approach for MPS I is compromised by the high frequency of graft rejection, incomplete donor chimerism and by inefficiency to prevent and correct skeletal abnormalities associated with the disease. This proposal aims at investigating if the use of supplemental stem cell therapy can improve the efficiency of HCT. Our research interest is focussed on a population of stem cells called mesenchymal stem cells (MSC), which can significantly contribute to regenerate tissues of the mesenchymal lineages, as stroma, bones and cartilage. We want to determine if MSC isolated from healthy donors may facilitate hematopoietic repopulation and skeletal tissues repair in a NOD/SCID/MPS-I mouse model. This initial experience

will serve to ascertain if our hypothesis is robust and meet all the criteria to transfer this therapeutic strategy into clinical intervention.

Dr. Richard Steet

University of Georgia Research Foundation, Athens, GA

Investigation of the cartilage pathogenesis of ML II and MPS”

While many tissues throughout the body are affected in individuals with MPS and MPS-related disorders, the central pathology in these patients is observed in bone and joints. Impaired development and progressive destruction of cartilage leads to many debilitating symptoms for MPS patients. The series of molecular events that lead from the primary genetic defect of MPS disorders to the characteristic bone and cartilage pathologies remains poorly understood. Defining these molecular events is important since it will point to new ways to treat these diseases without having to replace the defective enzymes or genes. Our laboratory has been using the zebrafish model system to study the cartilage defects associated with the MPS-related disorder, mucopolidosis II (ML-II). Our current evidence suggests that these cartilage defects are accompanied by changes in the expression level of several types of proteases, enzymes that can degrade extracellular proteins and cause damage to cartilage. We are planning to use zebrafish models of selected MPS and MPS-related disorders to directly test the role of these proteases.

These studies will provide new insight into the disease process of MPS disorders and will serve to identify new targets for therapy.

1st Year Research Reviews - 2008

Dr. Sara Cathey

Greenwood Genetics Center, North Charleston, SC

Natural history study for mucopolidosis

One year partnership grant with ISMRD.

Received 7-09

The Mucopolidosis Project of the Greenwood Genetic Center (GGC) began in 2005. The goals of the project are to establish databases of clinical and laboratory information about ML II and ML III alpha/beta, understand the disease course, provide natural history information to families and the medical community, and raise awareness of the mucopolidoses. These goals are being met and exceeded!

The largest group of individuals with ML II and ML III alpha/beta ever reported has been described in the medical literature. Families and doctors have a resource for reliable information. Scientists have access to samples for research. The ML Project is ongoing, allowing the natural course of the diseases to be more fully understood. Fifteen families attended the first ML Clinic at GGC in 2006. The majority of those families, plus others, will participate in GGCs Second ML Clinic, July 27-29, 2009. This is an opportunity to evaluate individuals over time. We can not only study disease progression, but also assess the impact of interventions. Understanding the natural course of disease builds the framework for successful treatments. What interventions work best, and when? Are there markers? of disease in the blood or urine that can be followed to guide therapies? Who are the best candidates for surgery? Which surgeries?

By supporting work like the ML Project, the National MPS Society continues to foster research, progress, and hope. Families remain the driving force of this incredible momentum. Your support and participation make great things possible.

Nicola Brunetti-Pierri, M.D.
Department of Molecular and Human Genetics
Baylor College of Medicine, Houston, TX
HDAd gene therapy for lysosomal storage disorders?
Received 7-09

Correction of the neurological manifestations of lysosomal storage diseases has been elusive so far. The goal of our project is to develop a safe and effective strategy for correction of the central nervous system manifestations of Mucopolysaccharidosis II which could be potentially applicable to other lysosomal storage diseases as well.

Helper-dependent adenoviral (HDAd) vectors are devoid of all viral genes and result in long term transgene expression in the absence of chronic toxicity. Because of their ability to infect nondividing cells, including cells of the central nervous system, HDAd vectors are particularly attractive for brain-directed gene therapy. Using a vector expressing a reporter gene (the green fluorescent protein), we have showed that HDAd vectors administered via intratecal injection into the cerebrospinal fluid (CSF) resulted in minimal systemic toxicity and extensive transduction of neuroependymal cells as well as neuronal cells. Importantly, the expression has lasted for at least 3 months and we are planning to look also at longer time points (6 months- 1 year). Given the encouraging results obtained with the reporter gene, we are performing experiments in a mouse model of Mucopolysaccharidosis II injecting a vector encoding for the therapeutic gene. These experiments are crucial because they will clarify whether our approach will allow the correction of the central nervous system manifestation in the mice affected with Mucopolysaccharidosis II.

Brett E. Crawford, Ph.D.
Zacharon Pharmaceuticals Inc., La Jolla CA, 92037
“Glycosaminoglycan inhibitors as substrate reduction therapies for MPS II”
Received 7-09

Thanks to the support of the National MPS Society, we have made important progress during the first year of our research project. As proposed, our work has focused on two aspects of developing a substrate reduction therapy for MPS II: i) the development of methods to quantify GAG accumulation in cultured human MPS cells and ii) the testing of candidate GAG inhibitors in this model system.

In order to develop methods to quantify GAG accumulation in cultured MPS cells, we obtained cells from patients with MPS II from the National Institute of General Medical Sciences Human Genetic Cell Repository. With these cells we developed improved methods to quantify GAG accumulation in cultured MPS cells (the Sensi?Pro Substrate Assay). The Sensi?Pro assay was validated by successfully detecting reductions in GAG accumulation in MPS II cells treated with recombinant iduronate sulfatase. By developing and validating these methods, we now have an experimental model to test the effectiveness of candidate drugs by measuring their ability to impact GAG accumulation in MPS cells.

We have recently found that the assay could also be used clinically as it has the sensitivity to detect GAG accumulation in urine, serum, and cerebral spinal fluid of patients with MPS with several advantages over the alternative methods. Using the Sensi?Pro assay, we have screened our candidate GAG biosynthesis inhibitors to determine if any of these compounds reduce GAG accumulation in MPS II. Through these studies we have identified a series of compounds that reduce GAG accumulation in MPS II cells.

These exciting and positive results provide a foundation to accomplish the next stage of the research project: testing the most promising candidate drugs in the mouse model of MPS II. We have obtained the MPS II mouse model (strain courtesy of Joseph Muenzer, MD, PhD) and are well positioned to accomplish the next stage of the research project.

Dr. Andrea Ballabio

TIGEM (Telethon Institute of Genetics & Medicine), Naples, Italy

Modulation of autophagy as a potential therapeutic approach for MPS?"

Received 7-09

We recently generated new insights in the pathogenic mechanisms of mucopolysaccharidoses and more in general of lysosomal storage disorders. We demonstrated that a crucial lysosomal degradative pathway, autophagy, is severely impaired in two mouse models of mucopolysaccharidoses, Multiple Sulfatase Deficiency (MSD) and mucopolysaccharidosis type-III A (MPS-III A). As a consequence of a defect of autophagy we observed an abnormal accumulation of several autophagic substrates such as polyubiquitinated aggregates, p62 protein and dysfunctional mitochondria, that lead to cell death and tissue pathology in both models analyzed. During the first year supported by MPS funding, we began to evaluate the therapeutic effect of preventing/removing autophagic-dependent accumulation of toxic substrates in MSD and MPS-III A mouse models. We used both a drug delivery approach based on rapamycin administration and a genetic approach based on crossing affected mice with p62 KO mice.

Newborn (0-2 gg) and adult (3 weeks of age) MSD and MPS-III A mice received intraperitoneal injection of rapamycin (20mg/kg) three times a week during all treatment period. Mice were analyzed at two time points: 1) an early time point (5 weeks of age) for biochemical and histological analysis (both MPS-III A and MSD mice) and 2) a late time point for biochemical, histological and behavioral analysis. We chose 20 weeks of age as late time point for MPS-III A mice and 12 weeks of age as a for MSD mice because the most significant difference between normal and affected mice in terms of behavior were observed at this age.

The first group of treated mice (either newborn- or adult-treated mice) were analyzed at 5 weeks of age (early time point) and collected tissues (brain and liver) assessed for the accumulation of p62, alfa-synuclein and polyubiquitinated proteins. In all affected mice (MSD and MPS-III A) the injection with rapamycin resulted in a significant decrease of toxic accumulation when compared to control PBS-injected affected mice. Importantly, the extent in the decrease of toxic accumulation was higher in mice treated at birth compared to mice treated at 3 weeks of age. We are now evaluating mitochondrial dysfunction and apoptotic cell death in the same tissues. These first data are very encouraging. The other experimental groups of mice (MSD and MPS-III A mice injected with rapamycin along with control normal and affected mice PBS-injected) will be analyzed at 20 weeks (MPS-III A) or at 12 weeks of age (MSD) to evaluate if the rapamycin treatment is able to recover a normal behavior in affected mice.

We plan to complete this task within 1 year.

The conditional p62 KO mice have been received from Dr Tanakas group (Laboratory of Frontier Science, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; Komatsu et al., 2007). These mice are now available in our animal facility and the colony has been established and expanded. These mice will be crossed with either MSD or MPS-III A mice. We plan to induce the ablation of p62 in specific tissues (brain and liver) to evaluate to which extent the abnormal accumulation of this substrate is involved in the tissue pathology and consequently if its ablation

could prevent the pathology itself.
We plan to complete this task within 1 year.

Brian Bigger PhD

MPS Stem Cell Research Group

University of Manchester, Royal Manchester Children's Hospital

The effect of heparan sulphate on stem cell homing and engraftment in MPS"

Received 7-09

Heparan Sulfate: A Helping Hand or A Sticking Point?

Heparan sulfate is stored in the cells of MPS I, II, III, and VII patients. In unaffected cells, heparan sulfate has a very important role in cell to cell signalling: allowing cells to talk to each other by interacting with molecules on the surface of nearby cells, or released into the space between cells. Like all the glycosaminoglycans or GAGs stored in the MPS diseases, it consists of a protein core with large chains of sugars attached to it. The sugar chains can have sulfate groups attached in a variety of patterns, which give them patches of negative charge. The patterns of sulfation differ between different kinds of cells and organs across the body. It is believed that these changing patterns of sulfation are important in deciding which signals get processed by the cell, and which signals get ignored.

This work focuses on MPS I Hurler, and bone marrow transplantation. When bone marrow is transplanted, the cells enter the blood stream, and need to find their way to the bone marrow by following signals coming from the bone marrow, like a homing beacon. It is known that heparan sulfate is involved in mediating the homing signal, and we are investigating whether this interaction is altered in MPS I Hurler, and whether this might be influencing the success of bone marrow transplantation for MPS I Hurler, and potentially other MPS diseases.

It is well known that large amounts of heparan sulphate and other glycosaminoglycans are stored inside the cells of MPS patients, but we wanted to prove that heparan sulphate is found on the outside of MPS I Hurler cells, where it could take part in signalling. The pictures below show an excess of heparan sulfate, (shown in green) on the surface of bone marrow stromal cells taken from mice with MPS I Hurler. Having shown that there is more heparan sulfate on the cell surface, specialised antibodies were used to show that this heparan sulfate had different sulfation patterns to heparan sulphate from unaffected cells. We found that MPS I Hurler heparan sulfate carries more of the type of sulfation that tends to lead to increased signalling. Indeed, we found that bone marrow cells could home across matrix (the material that fills the gaps between cells) containing MPS I Hurler heparan sulfate almost twice as well as across matrix from unaffected cells. In summary, so far we have found

MPS I Hurler bone marrow stromal cells accumulate heparan sulfate externally as well as internally,

Heparan sulfate from MPS I Hurler cells has a specific structure that favors homing to bone marrow and alters cell:cell signaling.

As this work continues, we hope to clarify the precise role of this altered heparan sulfate in the bone marrow homing and engraftment process, and find ways of manipulating this pathway to improve transplant success.

Adriana M Montano, PhD

Saint Louis University

School of Medicine - Dept of Pediatrics, St Louis, MO

Identification of genes for keratin sulfate biosynthesis: toward development of RNAi mediated

therapy?”

Received 7-09

Mucopolysaccharidosis IVA (Morquio A disease) is caused by the deficiency of the enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS). The lack of this enzyme leads to the accumulation of undegraded glycosaminoglycans (GAGs): keratan sulfate (KS) and chondroitin-6-sulfate (CS) in all the cells of the body, especially in skeletal tissue.

Several treatment approaches have been focused on providing the deficient enzyme to Morquio A mouse models. Our approach is to partially block the synthesis of KS mainly in cartilage cells to stop the progression of the disease by attenuating one or more genes involved in the synthesis of KS (See the figure).

The complete set of genes involved in the synthesis of KS is unknown posing an obstacle for understanding the biochemical processes leading their accumulation. In order to overcome this problem, we have performed a detailed differential expression analysis and identified two candidate genes that may be involved in the synthesis of KS, and made progress towards their characterization. We have cloned the genes in an expression vector to study their effects in-vitro. In addition, we have cloned the genes in another vector that will facilitate the purification of the proteins resultant as the product of these genes. In-vitro analysis and the protein purification experiments are in progress. After the completion of these crucial experiments, we will focus, in the second year of our program, on (i) the suppression of these genes at the RNA level, and (ii) performing in-vitro tests in cartilage cells of Morquio A patients. The success of the second phase will provide a promising therapy technique for MPS IVA patients.

Mark S. Sands, Ph.D.

Washington University School of Medicine

Department of Internal Medicine, St. Louis, MO

Metabolic adaptations and phenotypic consequences of blocking lysosomal recycling?”

Received 7-09

Specific Aims:

- 1) Determine the effects of reduced lysosomal recycling on the energy imbalance in affected cells.
- 2) Determine the effects of dietary intervention on the progression of disease.

Summary:

We generated preliminary data showing that there was a significant energy imbalance of unknown etiology in 5 murine models of different lysosomal storage disease (Woloszynek et al., 2007, J. Biol. Chem., 282:35765). The energy imbalance manifests as a significant decrease in adiposity. These data suggest that lysosomal storage disorders are diseases of deficiency as well as excess (lysosomal storage). We hypothesized that the energy imbalance was due to an increase in energy-intensive reactions required to maintain homeostasis in the context of a decreased pool of metabolites from lysosomal recycling. The goal of this research was to provide additional data supporting this hypothesis and determine the effects of an energy-rich diet on disease progression. We performed an unbiased metabolomics survey of the liver of MPS I animals. This analysis showed that simple sugars, nucleotides and lipids were decreased in the MPS I liver compared to normal. This supports the hypothesis that the animals are in a state of deficiency. The metabolomics analysis also showed an increase in amino acids, amino acid derivatives, dipeptides, and urea. These data suggest that increased protein catabolism is at least partially fulfilling intermediary metabolism. When MPS I animals were placed on a high fat, simple sugar diet (high fat diet) for 4 weeks, most of the abnormal metabolite levels approached normal. Consistent with the apparent increase in protein catabolism, we observed an increase in a marker of autophagy (LC3) in the livers of both MPSI and MPS VII animals. Autophagy was decreased in the livers of MPS I animals

placed on the high fat diet, however, the decrease was not statistically significant ($p=0.08$). Interestingly, autophagy was significantly reduced and approached nearly normal levels in the livers of MPS VII animals maintained on a high fat, simple sugar diet from weaning. MPS VII animals maintained on the high fat diet from weaning had significantly increased body weight for the duration of the study (7 months). Unfortunately, there was no significant improvement in longevity, retinal function or cardiac morphology or function. These data strongly support the hypothesis that animals with LSDs are suffering from nutritional stress similar to starvation. Although a crude high fat, simple sugar diet resulted in only minimal clinical improvement, a more targeted nutrient approach may yet prove beneficial as an adjunct therapy as the mechanism of this energy imbalance is better understood. The data generated from this MPS Society-funded project is currently under review at the Journal of Biological Chemistry.

Year 2 funding:

We have made significant progress on this important area of research during the first year of funding. We are currently breeding the MPS I and MPS VII mutations onto the ATG5- and ATG6 (Beclin)-deficient animals. These gene knockouts interrupt normal autophagy. These double mutant animals will allow us to determine if the changes in autophagy in response to the energy imbalance are helpful or harmful.

Marta Serafini, PhD

**STEMMPS Unit, Dulbecco Telethon Institute at M.Tettamanti Research Center Clinica
Pediatria Univ. Milano-Bicocca, Monza, Italy**

“Marrow mesenchymal stem cell therapy for MPS I?”

Received 7-09

The goal of our project is the identification of a novel cell therapy strategy capable of effectively alleviating disease manifestations, in particular at the skeletal level, still affecting MPS-I children after allogeneic hematopoietic stem cell transplantation.

To reach this goal, we are focusing on the following different aspects:

1. Optimize the culture of human mesenchymal stem cells (hMSC), a population of multipotent stem cells with interesting therapeutic potential and compare hMSC derived from healthy and MPS-I children of similar age.

We defined a new protocol to isolate hMSC clones and compared the properties of our cultures kept under hypoxic/normoxic conditions. We focused on the establishment and characterization of MSC lines from healthy young donors (age 1-10) and MPS-I patients (age 1-3). Lines have been maintained in long-term in vitro culture. We are particularly interested in the capacity of these cells to differentiate into the osteogenic lineage, as our approach aims to demonstrate the repair of MPS-I problems at the skeletal level. For this reason, we compared the in vitro osteogenic differentiation of the generated populations in a time course, monitoring the formation of mineralized matrix and the osteogenic transcription profile. The comparison of osteogenic properties between cells derived from healthy donors and MPS-I patients did not reveal significant differences. Ongoing experiments will evaluate within a few weeks if both the populations are able to establish the bone microenvironment in vivo.

2. Explore the potential of umbilical cord blood (UCB-MSC) and amniotic fluid (AF-MSC) as alternative sources of hMSC,.

Few reports have described an advantage of fetal hMSC in osteogenic differentiation potential over adult hMSC, which may represent optimal sources for bone repair and regeneration. We are currently working on isolation procedures to improve the success rate, which is 20% for UCM samples and 60% for AF samples. We fully characterized the populations obtained and compared their capacity to differentiate into mature osteoblasts and produce a mineralized matrix under permissive osteogenic conditions. The next step of our work will be the evaluation of our transplant experiments in mice to assess their osteogenic capacity.

3. Optimization of hMSC transduction with lentiviral vectors (LV).

To track hMSC transplanted in the NOD/SCID/MPS-I mice, we inserted the GFP reporter gene into hMSC. For this reason, GFP-transduction of hMSC was optimized, tuning vector dose and number of rounds of transduction. According to our generated protocol, we genetically modified the cells with an HIV-1-based lentiviral vector carrying the eGFP reporter gene (in collaboration with Dr. A. Biffi). Efficient GFP expression (range: 87-95% GFP positive cells) was observed three days after transduction and the percentage of GFP-expressing cells remained virtually unchanged in subsequent 10 passages, which correlated to 35 to 40 days post-transduction. We observed that LV-transduced hMSC have a lower proliferation capacity compared to untransduced cells.

To further evaluate whether lentiviral transduction altered the differentiation properties of hMSCs in vitro, we analysed the cells for the typical markers CD73, CD105, CD90 and CD146. We also induced the transduced cells to differentiate into cells of the mesenchymal lineages: adipocytes, osteocytes and chondrocytes. The data obtained are comparable with those from untransduced hMSC. In addition, chromosomal stability of the transduced cells was confirmed by karyotype analysis.

4. Establishment and optimization of the transplant protocol to evaluate the contribution of hMSC to MPS-I disease model.

We are comparing different transplantation protocols in the mouse model MPS-I in order to identify the one allowing for efficient MSC homing to the skeleton and phenotypic amelioration. In fact, for MSC trafficking experiments, the timing of delivery, number of cells delivered and site of MSC infusion may impact the engraftment efficiency and the destination of exogenously delivered cells. Completion of the work dedicated to the assessment of the feasibility and therapeutic efficacy of MSC transplantation in MPS-I mice is expected in the next few months. Through a direct collaboration with Dr. Gupta, we are scheduled to begin the transplant experiments in September 2009.

Richard Steet, Ph.D.

Assistant Professor of Biochemistry and Molecular Biology Complex Carbohydrate Research Center

University of Georgia Research Foundation, Athens, GA

Investigation of the cartilage pathogenesis of ML II and MPS?"

Received 7-09

The pathogenic mechanisms that underlie the bone and cartilage phenotypes in MPS and MPS-related disorders are only now beginning to emerge. Defining these mechanisms is important since it will potentially aid in the development of new therapies. Our proposal aims to take advantage of the power and speed of the zebrafish system to identify factors that contribute to the cartilage pathogenesis in mucopolysaccharidosis-II and directly test their contribution to the disease process. These studies will utilize our newly characterized zebrafish model for ML-II and will serve as a prelude

for therapeutic development. We are also attempting to model additional MPS and MPS-related disorders in this model organism in order to better understand how loss of specific hydrolases results in the phenotypes noted in patients.

Over the past year, we have analyzed the gene expression profiles in wild type and ML-II whole zebrafish embryos and isolated zebrafish chondrocytes, demonstrating that several genes relevant to bone and cartilage homeostasis appear to be upregulated, including cathepsins and matrix metalloproteinases. These findings have been confirmed in chondrocyte and fibroblast-like synoviocyte samples from the feline ML-II model. Unlike MPS cartilage and synoviocyte cells, however, we have not seen increases in genes that express pro-inflammatory cytokines. These preliminary findings may suggest unique mechanisms of cartilage damage in ML-II that are independent of inflammation. Our goal in the coming year will be to reduce the expression of the upregulated proteases in the ML-II zebrafish and assess whether this reduction can suppress the cartilage phenotypes in our model. We are also actively investigating the mechanisms that lead to upregulation of these potentially damaging proteases.

Attempts have been made to generate models for other MPS disorders using the zebrafish system. As an entry point to this work, the activity of the GAG-degrading enzyme, beta-glucuronidase, was depleted in zebrafish embryos using antisense oligonucleotides called morpholinos. Although we were able to effectively suppress beta-glucuronidase activity to less than 5% of wild type levels, no obvious phenotypes were observed in these mutant embryos. This finding has prompted us to examine the lysosomal biology of zebrafish in greater detail. To do so, we determined the expression (by RT-PCR) and the activity (using fluorescent substrates) of several lysosomal hydrolases across a developmental timeline (0-5 days post-fertilization). Our results indicate that, unlike enzymes involved in the breakdown of protein-bound oligosaccharides, expression of GAG-degrading enzymes - such as alpha-iduronidase and beta-glucuronidase - does not significantly increase until later developmental stages (4-5 dpf). By this stage, many of the vital organ systems have already developed in the embryos. This fact, along with the possibility that GAG accumulation and turnover is relatively slow in developing zebrafish tissue, may limit our ability to model certain MPS disorders in this organism using our current antisense-based approach. Thus, we are now actively seeking new ways to genetically target these hydrolases in order to generate zebrafish mutants with sustained loss of enzyme activity.

2nd Year Research Reviews - 2008

2008 Second year reviews

In 2008 the National MPS Society awarded two MPS II grants, one MPS II grant and five general MPS research grants.

Dr. Nicola Brunetti-Pierri
Telethon Institute of Genetics and Medicine
Naples, Italy

HDAd gene therapy for lysosomal storage disorders

Correction of the neurological manifestations of lysosomal storage diseases has been elusive so far. The goal of our project was to develop a safe and effective strategy for correction of the central nervous system manifestations of MPS II which could be potentially applicable to other lysosomal storage diseases as well.

Helper-dependent adenoviral (HDAd) vectors which are devoid of all viral genes can infect post-mitotic cells, including cells of the central nervous system (CNS) and are particularly attractive for brain-directed gene therapy. We have showed that intrathecal injections of HDAd resulted in extensive transduction of ependymal cells and sustained expression of the transgene up to one year post-administration. We have also demonstrated, for the first time, the ability of HDAd injected by this route of delivery to transduce neuronal cells. The transduced neuroepithelial cells can be potentially used to secrete therapeutic proteins into the cerebrospinal fluid (CSF) and provide them via cross-correction to non-transduced cells. Targeting of neuronal cells and long-term transgene expression make this approach attractive for the treatment of several neurologic diseases, including lysosomal storage disorders. An HDAd vector encoding the iduronate sulfatase has been injected by intrathecal injection in a mouse model of Mucopolysaccharidosis II and we are currently processing brain samples to investigate whether correction of central nervous system manifestations has occurred.

Dr. Brett E. Crawford

Zacharon Pharmaceuticals Inc., La Jolla CA,

Glycosaminoglycan inhibitors as substrate reduction therapies for MPS II

With the support from the National MPS Society, we have made significant progress toward developing a new approach designed to treat both the neurological and non-neurological symptoms of MPS. This approach is based on drugs which modify GAGs so that the deficient enzyme is not required to degrade them. Our progress has moved through three important stages of drug development: i) development of methods to quantify disease in human MPS cell samples, ii) testing of candidate drugs in this model system, iii) testing of candidate drugs in mouse models of MPS. The following is a brief description of our progress:

Development of methods to quantify disease in cultured human MPS cells.

The first goal of our proposed research was to develop a laboratory system based on cells from patients with MPS that would allow us to test potential treatments in the lab. A testing system like this is essential to discovering new drugs which can modify GAGs so that the deficient enzyme is not required to degrade them. We were very successful in this aim with the development of the SensiPro Assay. This assay can sensitively and specifically measure the amount of GAGs that have accumulated in the lysosomes of MPS patient samples or cells. While we originally developed the SensiPro Assay to guide our own drug development efforts, we found (through a series of collaborations with MPS experts) that the assay was also very useful in other ways. We have found that it can also detect MPS disease in urine, tissue, blood, and cerebrospinal fluid in addition to human cells. The assay has also been found to be very useful in measuring treatment response, thus it can help in the development of other new treatments and can help clinicians monitor MPS patients and optimize treatment decisions. We are also conducting experiments to show how the assay could be used as a newborn screen for MPS.

Testing of candidate treatments in the SensiPro Assay: With this testing system developed, we then tested over 100,000 drug candidates and found several promising drug candidates which reduce the lysosomal accumulation of GAGs in MPS I, II, and III (A, B, and C). This exciting milestone demonstrated the principle of modifying GAGs so that the deficient enzymes are not required to degrade them. In order to move these drug candidates toward clinical testing, we first need to improve their characteristics. To accomplish this, we began synthesizing and testing a large number of derivatives of the drug candidates. Through this ongoing effort, we have improved the characteristics of the drug candidates, and the most effective candidates are currently being evaluated for properties that are required for a safe and effective therapy.

Testing of drug candidates in mouse models of MPS: We obtained the mouse models of MPS I, II, and IIIA. We originally proposed to test our compound in the MPS II mouse, but our MPS II mouse

colony is not large enough yet for testing drug candidates. Because our treatment is also effective in MPS IIIA, we tested the most promising drug candidate in mice with MPS IIIA. While this initial study is small, we were very excited to find that the lysosomal accumulation of GAGs was reduced in the brains of the MPS IIIA mice. Also, the mice displayed no adverse effects from treatment over the 20 day study. Larger efficacy studies in MPS I, II, and III are planned for the near future. We are also happy to report that we have recently been awarded an NIH Small Business Innovative Research grant to further our efforts to develop a new approach to treating MPS. We are thankful for the ongoing support of the MPS Society which is helping fund the development of this new approach to treating MPS.

Dr. Andrea Ballabio

TIGEM (Telethon Institute of Genetics & Medicine)

Naples, Italy

Modulation of autophagy as a potential therapeutic approach for MPS

Recent studies highlight the prominent role of inefficient autophagic-dependent mitochondria dysfunction in the pathogenesis of neurodegenerative autophagic diseases as Huntington.

Mitochondria are organelles recognized as central players in cell death. Proper recycling of dysfunctional mitochondria relies on PINK1/Parkin -dependent selective autophagy (a process known as mitophagy) that avoids the release of pro-apoptotic factors.

These studies prompted us to investigate the role of mitochondria in the pathogenesis of MPSs.

Our recent findings demonstrated that dysfunctional mitochondria accumulate, together with other toxic substrates such as p62-aggregates and poly-ubiquitinated proteins, as a consequence of autophagic stress in two mouse models of mucopolysaccharidoses, MSD and MPS-IIIa.

During the second year of MPS funding we decided to investigate more in depth the role of the lysosomal-mitochondrial axis dysfunction in the cellular pathology of both MSD and MPS-IIIa mice. Moreover, as planned in our proposal, we evaluated the recovery of normal mitochondrial function in the brain and liver of MSD and MPS-IIIa mice treated with rapamycin.

We observed that dysfunctional mitochondria accumulate, in a different fashion, in liver and brain from the MPS mouse models analyzed. These organelles showed reduced membrane integrity, low ATP content and significant morphological changes (being fragmented in brain and giant in liver). Such morphological/functional changes lead to cytochrome c release and apoptotic cell death in the liver of the affected mice. We also analyzed the mitochondrial function in the MSD and MPS-IIIa mice treated with rapamycin. After 5 weeks of treatment we observed a significant reduction of dysfunctional mitochondria together with a partial rescue of the pathological phenotype in treated mice. We have obtained the following results:

- 1) The mitochondria recovered a normal morphology in affected mice treated with rapamycin.
 - 2) We did not detect any release of cytochrome c from liver tissue of affected mice treated with rapamycin, thus meaning that rapamycin successfully enhances the clearance of damaged mitochondria.
 - 3) We have also detected a reduction in the levels of pro-inflammatory cytokines in treated mice. This could be due to either the removal of toxic substrates (aggregate proteins and dysfunctional mitochondria), or to rapamycin immunosuppressive activity, or both.
- Together our data provide new evidence that the mitochondrial-lysosomal axis differentially contributes to neurodegeneration and to the systemic features in severe models of mucopolysaccharidoses and that induction of autophagy could be a feasible therapeutic approach for this type of inherited disorders. Importantly, our data need to be extended to evaluate whether the rapamycin treatment was indeed effective in rescuing neurodegenerative processes and behavior phenotype in the affected mice.

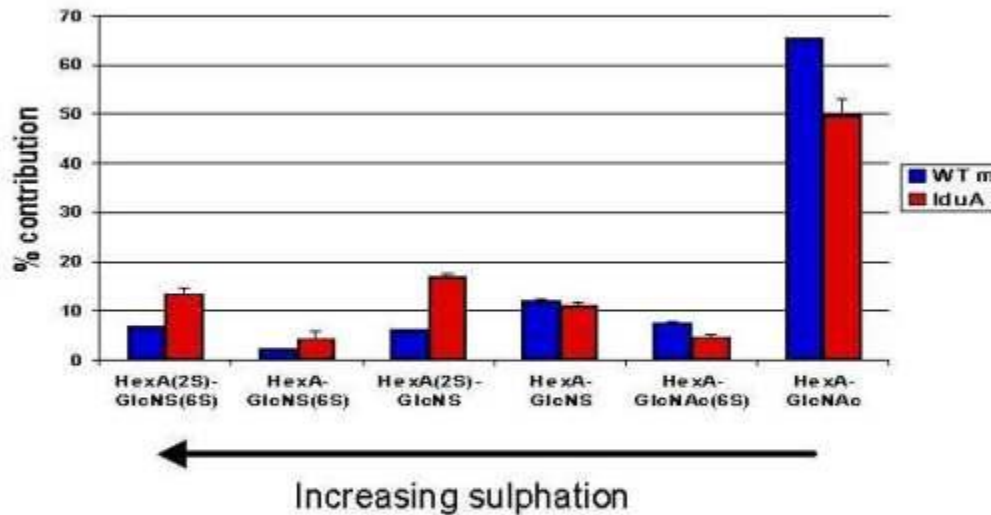
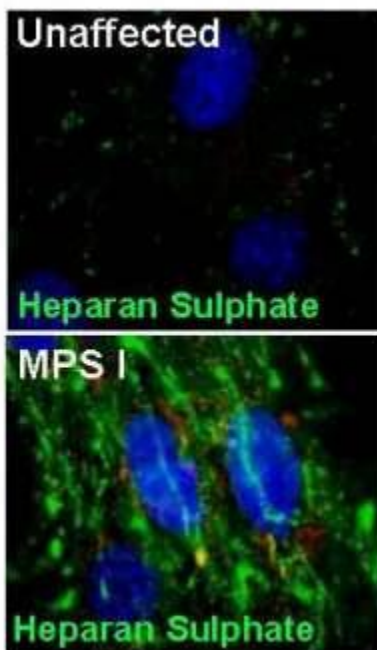
Dr. Brian Bigger
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The effect of heparan sulphate on stem cell homing and engraftment in MPS I

Introduction

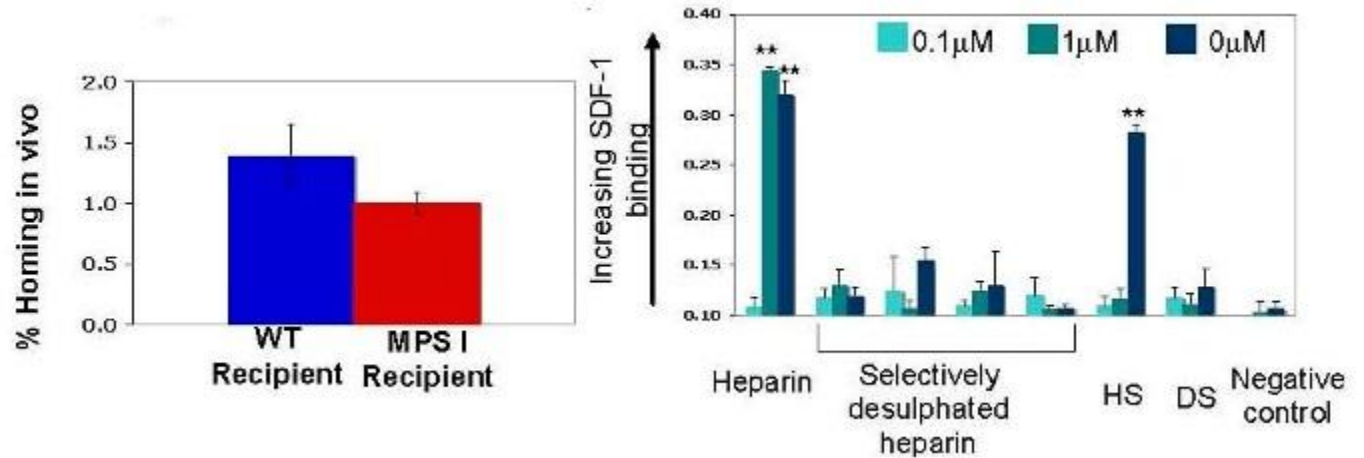
When stem cells are transplanted into patients, they must find their way from the bloodstream into the bone marrow by following signals called chemokines, which are produced by the bone marrow. In recent years, it has become clear that chemokines interact with glycosaminoglycans, heparan sulphate in particular, in order to function normally. Heparan sulphate (HS) is one of the stored molecules in MPS I, as well as MPS II, MPS III and VII. Here, we examine how HS accumulation in MPS I could be affecting homing of stem cells to the bone marrow during bone marrow transplantation, and thus contribute to graft failure.

Year 1: MPS I Bone Marrow Cells Accumulate Excess Highly Sulphated Heparan Sulphate



We demonstrated that MPS I bone marrow cells do not only store HS inside the cells, but outside cells in the extracellular matrix (ECM). The ECM is a sticky material made up of proteins and sugars which anchors and displays chemokines for interaction with homing cells. In the photographs (above left), heparan sulphate is stained fluorescent green, and there is clearly more associated with MPS I cells (bottom image). In the graph (above right), each bar represents the percentage of disaccharide building blocks that have sulphate groups. MPS I bone marrow has a dramatic increase in sulphated disaccharides in HS. This shows that MPS I HS is much more highly sulphated than normal. The amount of sulphation is important in chemokine-HS interactions.

Year 2: High Levels of Highly Sulphated Heparan Sulphate are Inhibitory to Stem Cell Homing



Experiments in vivo showed decreased homing in MPS I recipients of bone marrow transplants (above left). Work in vitro showed that heparan sulphate decreased the ability of bone marrow stem cells to home to the chemokine SDF-1, which is the most important chemokine for bone marrow transplant. This work showed that at low concentrations, the inhibition of homing was not dependent on the sulphation state of HS, however at high concentrations, sulphation state became important. A binding experiment (above right) showed that this was because SDF-1 will only bind to highly sulphated forms of HS at high concentrations it does not bind to desulphated forms at all.

Conclusions

We concluded that the decreased homing seen in transplants to MPS I recipients was caused by the unique combination of HS that is both highly sulphated and in massive excess in MPS I bone marrow cells, which causes SDF-1 to become trapped in super-sticky ECM, preventing it from signalling to transplanted cells. This is a disease-specific defect in homing that could be contributing to graft failure in the clinic.

Awards

This work was presented at the Harden Conference on Heparan Sulphate in Cambridge in 2009, where it was awarded the Mituzani Foundation Poster Prize. It was also selected for oral presentation at the Clinical and Laboratory Sciences Showcase, University of Manchester 2009.

Dr. Adriana M Montano

Saint Louis University School of Medicine
St Louis, MO

Identification of genes for keratin sulfate biosynthesis: toward development of RNAi mediated therapy

Study has extended to 2011

Dr. Mark S. Sands

Washington University School of Medicine
St. Louis, MO

Goals:

1) determine the effects of reduced lysosomal recycling on the energy imbalance in affected cells, and 2) determine the effects of dietary intervention on the progression of disease.

We previously showed that there was a significant energy imbalance in the form of depleted adipose stores in mouse models with lysosomal storage diseases (three of which were MPS disorders). We hypothesized that the energy imbalance was due to interrupted lysosomal recycling of macromolecules and the subsequent increase in energy expenditure required for the synthesis of new macromolecules. In order to further test this hypothesis we measured the levels of over 1,500 metabolites in liver homogenates from MPS I mice. Molecules directly involved in energy utilization such as simple sugars, nucleic acids and lipids were depleted. This is consistent with the animals being in a state of nutrient deprivation. Interestingly, the levels of amino acids, amino acid derivatives and dipeptides were increased suggesting that protein catabolism, perhaps due to increased autophagy, is at least partially fulfilling intermediary metabolism. We showed that autophagy is increased in the livers of both MPS I and VII mice. The initial observation of energy imbalance also led us to hypothesize that a diet rich in energy (simple sugars and lipids) would decrease the severity of the disease. Therefore, we put both MPS I and VII mice on a high fat, simple sugar diet. Although there was no significant increase in life span or retinal function, most of the abnormalities identified on the metabolomic screen approached normal levels. In addition, the animals were now able to increase their adipose stores and autophagy was reduced to near normal levels. These data support our hypotheses that interrupted lysosomal recycling leads to a significant energy imbalance and that nutritional intervention can ameliorate some of the biochemical abnormalities associated with these diseases. This work was recently published [Woloszynek JC, et al., (2009) *J. Biol. Chem.* 284:29684-29691].

This grant also partially supported a study in which we combined adeno-associated virus (AAV)-mediated, CNS-directed gene therapy with bone marrow transplantation (BMT) in the murine model of MPS IIIB. We hypothesized that systemic therapy (BMT) would synergize with CNS-directed gene therapy to increase efficacy in MPS IIIB which has been relatively refractory to most therapies. CNS-directed, AAV-mediated gene therapy alone provided the greatest clinical benefit. The addition of BMT added little in the way of efficacy and actually decreased the life span slightly. This study was recently published [Heldermon C, et al. (2010) *Mol. Ther.* 18:873-880]. We have initiated another study in the MPS IIIB mouse where we combined CNS-directed AAV-mediated gene therapy with systemic lentiviral-mediated gene therapy. The rationale for this study is that the systemic lentiviral-mediated gene therapy would result in persistent high levels of circulating NaGlu activity that will enhance the effects of the CNS-directed therapy. It has been shown in another model of MPS that persistent high levels of circulating enzyme can result in some CNS correction. The preliminary data (14-16 months of age) suggest that the combination of AAV-mediated CNS-directed gene therapy combined with systemic gene therapy provides the greatest clinical benefit.

Publications:

Woloszynek JC, Kovacs A, Ohlemiller KK, Roberts M, Sands MS: Metabolic adaptations to interrupted glycosaminoglycan recycling. *J Biol Chem*, 284:29684, 2009.

Heldermon C, Ohlemiller KK, Herzog E, Vogler C, Qin E, Wozniak DF, Tan Y, Orrock J, Sands MS: Therapeutic efficacy of bone marrow transplant, intracranial AAV-mediated gene therapy or both in the mouse model of MPS IIIB. *Mol. Ther.*, 18:873, 2010.

Dr. Marta Serafini

Dulbecco Telethon Institute at M.Tettamanti Research Center Clinica Pediatrica Univ. Monza, Italy

Marrow mesenchymal stem cell therapy for MPS I

The overall aim of ongoing experiments on this project is to establish a new stem cell-based therapy to improve the outcome of hematopoietic cell transplantation with a particular emphasis on the skeletal defects affecting MPS-I patients. For this purpose, we have used two

complementary cell systems: on one side human mesenchymal stem cells (hMSCs) and on the other murine mesenchymal stem cells (mMSCs).

We have successfully isolated hMSCs from healthy donors and three MPS-I patients. We have evaluated their intrinsic osteogenic differentiative potential in vitro and, so far, our data indicate that genetically affected cells do not differ from healthy donors-derived cells, suggesting that microenvironment and/or signalling from neighbouring cells might be responsible for the observed phenotype at skeletal level.

In order to investigate the best source of stem cells, hMSCs have been isolated from healthy donors also from umbilical-cord blood, amniotic fluid and umbilical cord with an increasing efficiency in our system (around 70% for bone marrow and amniotic fluid, around 80% for digested umbilical cord and around 25% for umbilical cord blood). These cells differentiate in vitro in adipocytes, chondrocytes and osteoblasts and we are now evaluating their in vivo potential.

In parallel, we sought to isolate and characterise mMSCs. So far two protocols have been used and tested as it is widely accepted that mMSCs isolation protocols are less established. With our methods by the first passages we obtain a mixed population with an average of 50% of MSCs assessed by detection of markers as CD73, CD29, CD44, SCA1, CD36 and negativity for B220, CD11b and CD45 (by antibody staining), and good potential to differentiate in adipocytes, osteoblasts and chondrocytes. We are now in the process of isolating and culturing mMSCs using an inactivated feeder layer of murine fibroblasts, as a mean to increase the homogeneity and efficiency of mMSCs isolation.

In order to test the rescue capability of HCT with or without mesenchymal stem cells (MSCs), we planned to use the mouse model as our experimental system. To date, different murine models have been reported with mutations in the IDUA gene. Originally we established to use an immunodeficient mouse, the NOD/SCID/MPS-I (Garcia-Rivera et al., 2007) infused with human-derived MSCs.

Before starting the transplant experiments, we analysed the skeletal phenotype of this and other mutant models and we found that the range of bone-defects varied in penetrance and severity across the mutants. This situation could be compared to the differences observed in phenotype in MPS-I patients, representing a valuable tool to study the feasibility of MSCs transplant also in less severe forms of MPS-I.

In the last few months we have isolated GFP+ mMSCs and transplanted them into the different MPS-I mouse models. At defined time points after the infusion, we plan to evaluate the skeletal phenotype improvement, with or without hematopoietic stem cells. We have also tested several injection sites as MSCs are technically challenging to infuse for their intrinsic tendency to adhere. As demonstrated by our and other groups, intravenous injections are often lethal to the recipient animal, and cells tend to colonise the lungs and the heart but cells are rarely detectable in other inner organs and/or bones. We are now evaluating in site injections, intra-cardiac injections and possibly the use of cells containing-scaffold materials.

Dr. Richard Steet
University of Georgia Research Foundation
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Investigation of the cartilage pathogenesis of ML II and MPS

Defining the pathogenic mechanisms of MPS and MPS-related disorders is important since it will potentially aid in the development of new therapies. Our proposed research was designed to take advantage of the power and speed of the zebrafish system to identify factors that contribute to the cartilage pathogenesis in mucopolidosis II or ML-II, with the goal of directly investigating the contribution of these factors towards the disease process. With ongoing support from the MPS Society, we have now identified and confirmed that several enzymes - including cathepsins K, L and S and matrix metalloproteinase 13 - are upregulated in ML-II zebrafish embryos as well as feline ML-II tissues (Petrey A., Flanagan-Steet H., Nairn A., Moremen K., Haskins, M. and Steet R., manuscript in preparation). The activity of the cathepsins is elevated in ML-II zebrafish at time periods when only marginal activity can be detected in wild type embryos, suggesting that sustained or unregulated expression of these hydrolases occurs in response to impaired mannose phosphorylation. This abnormal expression may also compound the hypersecretion of these enzymes caused by defective mannose 6-phosphate dependent lysosomal targeting. Our current studies are focused on 1) localizing the increased cathepsin expression within specific tissues of our zebrafish model and 2) testing whether a reduction in this activity by genetic or pharmacological means will have therapeutic potential. In the coming years, we plan to translate any positive outcomes from our zebrafish experiments to studies in the feline ML-II model in collaboration with Dr. Haskins at the University of Pennsylvania.

Another facet of our MPS Society-funded project was to generate models for other MPS disorders using the zebrafish system, with the hope of using these models to explore whether common pathogenic mechanisms exist between ML-II and MPS disorders. Although we were able to reduce the activity of beta-glucuronidase (GUSB; the cause of MPS VII), in zebrafish embryos to less than 5% of wild type levels, no obvious phenotypes were observed. These data suggest that the relatively short duration of morpholino-induced gene suppression may limit our ability to model the more progressive MPS disorders in this organism. This finding led us to seek new ways to establish stable genetic zebrafish mutants with sustained loss of enzyme activity. In a fortunate respond to our request, GUSB was selected by an NIH-funded initiative that is tasked with generating zinc-finger nuclease constructs suitable for the stable suppression of genes in zebrafish. The development of a zebrafish model for MPS VII will be ongoing in the second half of this year in our lab and will expand the experimental systems available to investigate MPS pathogenesis.

As a new investigator, I am truly grateful to the MPS Society and the families for their support of this research project. Funding from the Society has proven vital in our ability to pursue higher risk experiments and we remain hopeful that the avenues of research that stem from this work can be translated into therapies for MPS and MPS-related disorders.