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Research From the Bedside to the Lab Bench & Back

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Micro-Series

KCUMB
KANSAS CITY UNIVERSITY OF MEDICINE AND BIOSCIENCES

Research from the Bedside to the Lab Bench & Back

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The goal of our research team is not to build better mice but to try to use these wonderful unique creatures to generate translational research to treat ill humans.

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Abstract

Transgenic mice represent a unique opportunity in biomedical research to discover the genes underlying disease and understand how manipulating the function of single genes and proteins alters physiology in a whole animal system. These advances in biomedical research may accelerate the time between when basic discoveries are made and when the research can be 'translated', that is, when the research will positively impact the lives of patients. The purpose of this article is to present some examples of promising mouse models of human diseases.

Introduction

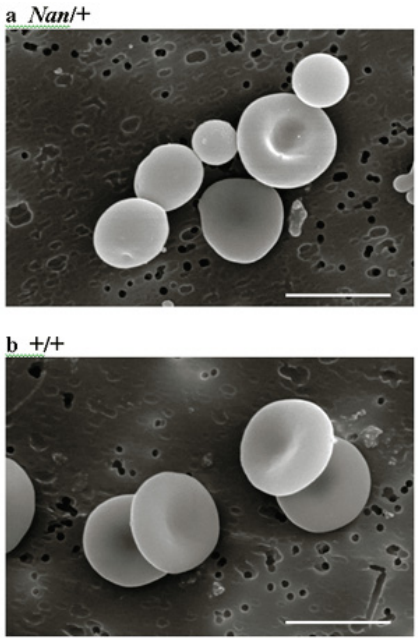
The interest of our research team at Kansas City University of Medicine and Biosciences (KCUMB) in Kansas City, Missouri encompasses studies that were generated by observations at the bedside which have led to clinically relevant laboratory biomedical research. Hopefully, the research will eventually return to the bedside in the form of improved treatment for human patients. This research involves a variety of human genetic diseases, most of which are rather common. In this research effort we believe that "the mice lead the way." Our research team has developed a program which is presently part of the translational research efforts at KCUMB and involves the use of mouse models as a foundation for identifying new causes of disease and perhaps the beginnings of novel therapies. The genetic diseases being worked on by our lab include: 1) the most common hereditary anemia in persons of northern European descent, hereditary spherocytosis; 2) the identification of a new pharmaceutical target to treat iron overloading in persons with genetic iron overload (hereditary hemochromatosis) and in patients with beta-thalassemia and sickle cell anemia who receive repeated blood transfusions for their treatment, and; 3) the identification of a new gene or pathway which can induce erythropoiesis. Erythopoiesis was uncovered by using a mouse model of a fatal hereditary anemia. Some mice survived and fully recovered from this deadly neonatal anemia to a normal hematological state by some unknown mechanism. In addition, there are several non-hematological studies underway including the development of gene therapy for treatment of Duchenne muscular dystrophy.

Hereditary Spherocytosis (HS): the Black Box

This hematological disease is the most common hereditary anemia in persons of northern European descent with an incidence as high as 1/2,000.2-4 The anemia is characterized by: 1) fragile, spherical red blood cells which exhibit osmotic fragility, 2) splenomegaly, and 3) anemia.

HS can be mild or severe resulting and life-threatening. The defects in these patients are in the cytoskeleton of RBCs which confer their stability and flexibility. After being released from the bone marrow, these RBCs must traverse the circulation at least a half million times in their lifetime. It is essential for their membrane cytoskeleton to have normal structure and flexibility. Many of the different genes that cause HS have been identified and they come in various flavors. However, the enigmatic black box of genetics in this patient population is that one-tenth of HS patients have no known genetic defect. [4] This puzzled researchers and stimulated research. The neonatal anemia mouse model (called the *Nan*, *N*eonatal *an*emia mouse) exhibits hereditary spherocytosis.[5] (See Figure 1.)

Figure 1
Spherocytic red blood cells seen in the Nan mouse, similar to that seen for human Hereditary Spherocytosis.



Mouse mutant loci are often named after the phenotype produced when genes are mutated. These mice imitate all of the clinical presentations of a typical HS patient but the gene causing this mouse mutant did not map to any of the known genes contributing to HS in mouse or humans. Therefore, this mouse provided an excellent opportunity of discovering a new gene for HS in mouse and perhaps in man. The gene defect we have discovered is not in a gene encoding a cytoskeleton protein (e.g. spectrin) but in a gene encoding an essential erythroid transcription factor. This genetic controlling element binds to DNA to turn on all the red blood cell cytoskeleton protein genes.[6] By analogy, it wasn't the light bulbs that were defective but the on-off switch that was discovered to be flawed. The gene's name was Kruppel-like factor-1 or *Klf1*.

Efforts are underway to examine HS patients who have no known gene defects. We have discovered two putative mutations in human *KLF1* in dominantly inherited HS patients which may be causative of their disease. Scientific proof of the contribution of these mutations to HS will be examined by the generation of transgenic mice expressing the mutant human form of KLF1 to show that expression of the mutant KLF1 protein leads to hereditary spherocytosis.

Iron Overloading: A Tale of Two Anemias, a Dickens of a Cloning Project

A challenge for clinicians is the treatment of patients with iron overloading. This can occur in patients with hereditary hemochromatosis, a disease in which there is uncontrollable iron absorption in the gut which leads to the deposition of iron in tissues and consequent end-organ disease. In many patients with beta-thalassemia or sickle cell anemia repeated blood transfusions alleviate disease symptoms but eventually lead to morbidity and mortality due to transfusion-dependent iron overload. To prevent this several iron chelators were developed and currently are being used. This represents a major achievement in translational medicine research.[7-8] At times problems with patient compliance arise as well as serious side effects from the use of these drugs including nephrotic syndrome.[9-11]

Once again, unusual mouse mutants (called the flaky skin mouse and the hereditary erythroblastic anemia mouse) provide a basis for novel therapy as these mice have been shown to excrete iron by some unknown mechanism.[12] The discovery of genes causing hereditary diseases often explains how those genetic diseases come about. Unfortunately, there are times when this is not the case.

Such is the case with the gene defect in the flaky skin and hereditary erythroblastic anemia mouse mutants. The mutated gene in these mice has an unusual name: *Ttc7*, tetratricopeptide repeat domain 7.[13] No one knows the function of the TTC7 protein. However, the identification and cloning of the gene(s) causing these two hereditary mice anemias may lead to novel pharmaceutical targets inducing urinary iron excretion as treatment for human iron overload.

Research is underway at our lab at KCUMB to identify the protein partners of TTC7 protein to determine the function of this unusual protein and learn how it can be exploited to treat human iron overload. We have discovered that the TTC7 protein has nuclear localization implying the function is involved in gene expression. This is an important way-step in translating our genetic mice research into a cure for human iron overloading.

Gene X: A New Erythropoiesis Factor

An additional mouse mutant used in our research laboratory is the X-linked anemia mouse, so named because it resides on the X chromosome. These mice exhibit a

severe, neonatal anemia with an unusual feature; the mice recover from their anemia shortly after weaning from their mothers.[14,15] (See Figure 2.) These mice have a mutation in a gene called *Gata1;* it binds to DNA of erythroid lineage genes at a site in the DNA where the nucleotide sequence GATA occurs in the promoters of these genes.[16] The production of RBCs absolutely requires the expression of GATA. The X-linked anemia mice have a mutation in which no GATA1 protein is made in half of its bone marrow cells. These GATA1 deficient cells are not expected to make red blood cells. However they unexpectedly gradually develop a compensatory effort that results in the production of RBCs. In hematology, this is like getting blood from a rock.

We surmised that there is another erythropoietic factor at work accounting for their recovery. Identifying this factor (which we call gene X) could result in treatment for conditions such as chemotherapy-induced anemia and is a major focus of our research team. This study involves the use of gene chip technologies in which the expression of genes in the spleens of *Xla* mice will be examined and compared to spleens from normal littermates to identify gene X. Our research team includes Michael Silvey, DO and research fellows from the Hematology Section at Children's Mercy Hospital (CMH) in Kansas City.

Together, we have found that the bone marrow of adult *Xla* female mice is hypoplastic and there is a concomitant enlargement of the spleen by 30-40% compared to normal female littermates. Neither normal or *Xla* adult female mice express *Gata1* mRNA in their liver. These surprising discoveries represent effective extramedullary erythropoiesis in the spleen and may reflect the site where the putative novel erythropoietic factor is to be identified.

Figure 2
The anemia of the Xla mouse. The pale anemia Xla mouse pup is seen on the right.

Figure 3
Xla is a transient anemia. Xla mice are severely anemic at birth, but are normal at about 3 weeks of age. ** - statistically significant differences = $p < 0.001$

	Genotype	Hb, g/dL	RBC count $x10^6/\mu L$	Hct (%)
2 Days**	*Xla*	6.7 ± 1.1	1.48 ± 0.23	18 ± 4
	(+/+)	13.7 ± 2.2	3.46 ± 0.35	44 ± 5
3 Weeks	*Xla*	11.7 ± 1.3	4.91 ± 0.48	39 ± 5
	(+/+)	12.0 ± 1.7	5.41 ± 0.46	41 ± 3

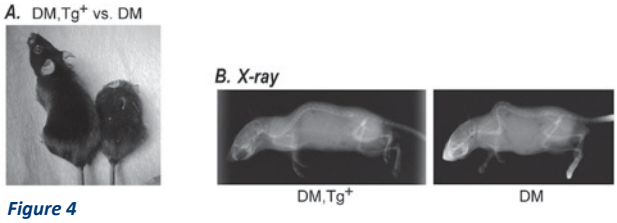
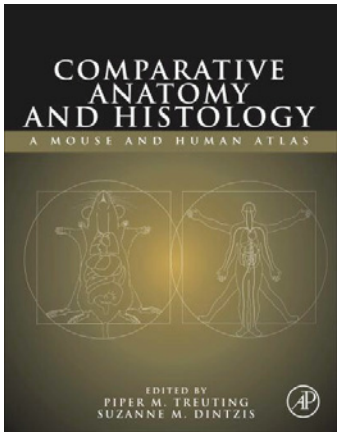


Figure 4
Rescue of the DMD model mice with expression of human retinal dystrophin expression in muscle. A. Male littermates both carrying the genetic defect leading to severe DMD. On the left is the DMD model mice with transgene = DM, Tg+ mice which had a normal lifespan. On the right is the DMD model mice = DM which suffered from a progressive, lethal myopathy and died at 3 months. B. The scoliosis occurring in DMD mice is not seen in DMD mice expressing retinal dystrophin (DM, Tg+).

Duchenne Muscular Dystrophy: The Eyes Have It

One of the most common neuromuscular diseases in humans is Duchenne muscular dystrophy (DMD) which is characterized by progressive muscle degeneration, loss of limb function and eventually, premature death. There is no cure for DMD. The genetic defect in these patients is in the dystrophin gene which directs production of the dystrophin protein which stabilizes muscle cell membranes. Its absence leads to the clinical presentation of DMD which is usually diagnosed at about three years of age. A clinical observation in the ophthalmology clinic at CMH led to the discovery of a "cousin" protein in the retina of the eye called retinal dystrophin. Our discovery of human retinal dystrophin led us to consider that this related protein, which is expressed mostly in the retina but not in skeletal muscle, might be a resource for gene therapy.[17]

Our research team includes CMH faculty Ann Modrcin, MD and Robert Rinaldi, MD who treat DMD patients. We postulated this eye protein might replace the missing skeletal muscle protein dystrophin. We created a human transgene to be expressed in a severe mouse model of DMD which closely mimics the human disease including the presentation of progressive severe muscle disease and premature death. In collaboration with Stephen Hauschka, PhD, at the University of Washington in Seattle our research team built a transgene that would express human retinal dystrophin in muscle. Basically, what was done was to replace the retinal on/off switch controlling expression of retinal dystrophin with a skeletal muscle on/off switch, namely a creatine kinase gene promoter.



Editor's Recommendation:

Comparative Anatomy and Histology: A Mouse and Human Atlas

by Piper M. Trueting, DVM, ACVP & Suzanne M. Dintzis PhD, Academic Press 2011

"Long overdue, the editors have assembled a vast array of knowledge, protocols, lab lore and practical advice in a concise, well illustrated, and easily accessible volume of practical comparative anatomy of the mouse and human. The strict inclusion of just these two species provides an important and practical simplification of critical issues in using the mouse to model human disease. This book is long overdue and much needed in any of the thousands of laboratories performing research with mice." (edited)

-Alexander D. Borowsky, MD., D.A.B.P., Associate Professor of Pathology and Laboratory Medicine, Center for Comparative Medicine, UC Davis, CA, USA

Consequently, retinal dystrophin would now be expressed in skeletal muscle and we investigated if it made a difference with the disease in the DMD model mice. The research team found that replacing skeletal muscle dystrophin with retinal dystrophin did alleviate symptoms of muscular dystrophy in the DMD model mice and changed a severe lethal muscle disease into a viable, mild myopathic disease. The DMD model mice did not die prematurely at three months of age but lived a normal lifespan and had a major reduction in their muscle pathology. In addition, the mice scoliosis, which is also typically seen in DMD patients, is rescued by the expression of retinal dystrophin (see Figure 4). The team is now examining ways to bring their findings to the bedside of patients.

Conclusion

We have presented several mouse models of human disease used in our lab that offer an unprecedented opportunity in biomedical research to understand how genes and proteins interact in a whole-animal system. These animal models offer unique insights that will accelerate the pace of discovery and ultimately, improve human patients' lives.

The goal of our research team is not to build better mice but to try to use these wonderful unique creatures to generate translational research to treat ill humans. Problems that occur at the sick human bedside demand solutions from translational researchers at the lab bench which can be brought back to the bedside to treat patients. Using a series of mouse mutants, our research team at KCUMB has been able to identify new pathways or genes which can be exploited to generate novel treatments for patients. The promise of discovery occurs when the mice lead the way.

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Disclosure

A relative of John C. Hagan, MD, III, Editor, wrote one of the chapters of sidebar textbook. MM