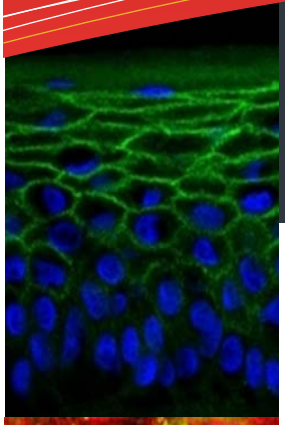
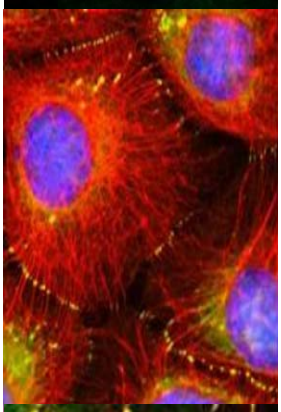
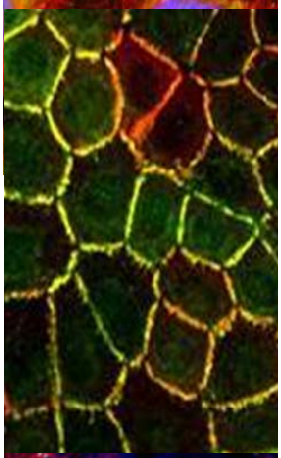
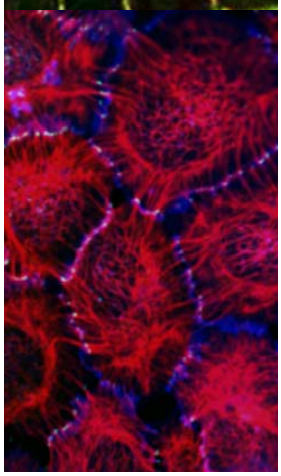
Northwestern University
Skin Disease Research Center

In this issue:

Volume 1
Issue 4
April 2013

PI Message

We've had another year of great expansion and support for NU researchers at the Northwestern SDRC. We've hosted or co-hosted 46 lectures from faculty or visiting professors during the past year through our Enrichment program - in addition to our continuing teaching of scientists through our Cores. We continue to grow our SDRC membership (now 67 members) - and are happy to include investigators who become interested in research related to epithelial cells and skin disorders. We highlight two of our newer members in this edition. Put the upcoming **NU-SDRC Research Retreat** on your calendars now -- **Tuesday, June 4th, 2013** from 8:30-11:00 in the Baldwin Auditorium followed by a poster session in the Ryan Auditorium lasting until 1 pm. Come by to hear about research that may give you some great ideas and exciting new collaborations -- and show your work at the poster session.

What's happening in the Cores? First of all, be aware of an upcoming move of all of our Service Cores from 13 Ward **to Tarry 4 in May 2013**. We will be hosting an open house shortly after this move, which will include Core demonstrations of new techniques and services.

We've expanded the capabilities of our **Keratinocyte Core** (which perhaps should be renamed Cultured Cell Core) – we are now able to grow for investigators primary keratinocytes, fibroblasts and melanocytes from both male and female donors of different ages, ethnicities and races. This is a marvelous opportunity to look at age-, sex-, ethnicity- or race-specific biological differences in cells. Keep in mind that we can simulate human epidermis (3-D organotypic rafts) with banked keratinocytes from patients with skin disorders or treat normal human keratinocytes biochemically or genetically to test drug delivery approaches, wounding, and the impact of intervention on human skin disease. Take a look at the article in this edition from Dr. Spiro Getsios, Director of the Keratinocyte Core.

He has treated keratinocytes with cytokines to create a 3-D psoriasis model and has shown that therapy with ephrins can prevent the cytokine-induced differentiation abnormalities. My own lab is currently using a variation of this psoriasis model to test the efficacy of siRNA spherical nucleic acids targeting receptors that are key for psoriatic responses and have similarly used the 3-D model to recapitulate a genetic disorder of keratin with patient keratinocytes, all grown from a single small punch biopsy of skin. In the near future, the Keratinocyte Core will be developing 3-D organotypic rafts that incorporate fibroblasts, melanocytes and perhaps other cells. We are also optimizing the xenografting of these 3-D rafts onto immunocompromised mice for long-term humanized skin studies. *What can you do with these models to study your*

Our **Pathology Core** is using new chromogens for immunohistochemical staining, such as the Vector Red Substrate rather than DAB for BrdU labeling; this chromogen is not only easier to see in tissues with melanin, but also fluoresces under fluorescent microscopy. The new Leica autostainer has significant cut down on processing time for our Core users. Particularly exciting this year is the expansion of our tissue bank, which has been tapped by scientists in many Northwestern departments. As Dr. Lavker, co-Director of Pathology, reports in this issue, archival tissue is available at short notice from a variety of common skin disorders - and a wide array of normal human skin specimens (similar to those available from the Keratinocyte Core). If you are interested in a disorder not listed herein, let us know. With our >100,000 specimens, we may well be able to find some paraffin-embedded tissue for you to use -- just pull down the request form from our website. We have also expanded our banked repository of frozen tissue from immune disorders - and normal skin. *Is your protein or gene of interest expressed in skin or altered in a skin disorders?*

The **DNA/RNA Delivery Core** now supports researchers in more departments than any other Core - and provides a unique service on campus with its generation of retro- and lentiviruses for both *in vitro* and *in vivo* applications. For example, the Core's products have facilitated the generation of inducible pluripotent cells (iPS cells) and, because they can be made at high titer, lentiviruses for protein and shRNA expression can be injected into the brain and other organs *in vivo* (see *The Latest News from the DNA/RNA Core*). The Core has helped to develop new materials for NU researchers, such as a panel of epithelial tumor cell lines stably expressing Tomato Red and luciferase, made in collaboration with the Tumor Biology Core in Evanston, and a new lentiviral expression vector for production and subsequent purification of proteins in mammalian cells through collaboration with the Center for Drug Design and Crystallography.

Finally, congratulations to our latest group of **awardees of the 2013-2014 Pilot and Feasibility Awards**: Jodi Johnson, PhD (Pathology Department): *Study of keratinocyte biology from kidney transplant patients with different skin types*; Thomas Meade, PhD (Chemistry Department): *Gold Nanoparticles Loaded with Cobalt Schiff Base-DNA Conjugates for Specific and Activatable Inhibition of Gli Transcription Factors*; Murali Prakriya, PhD (Molecular Pharmacology and Biological Chemistry Department): *Determining the role of CRAC channels for calcium dynamics and effector function in keratinocytes*; and Liang Zhou, MD, PhD (Pathology Department): *Functional Analysis of T Cell Transcription Factors in the Immune Regulation of Skin Inflammation*. Stay tuned for an upcoming mini-newsletter edition in which we will highlight these awardees and their plans for using the SDRC to address interesting research questions.

Hope to see many new SDRC Users this year.....

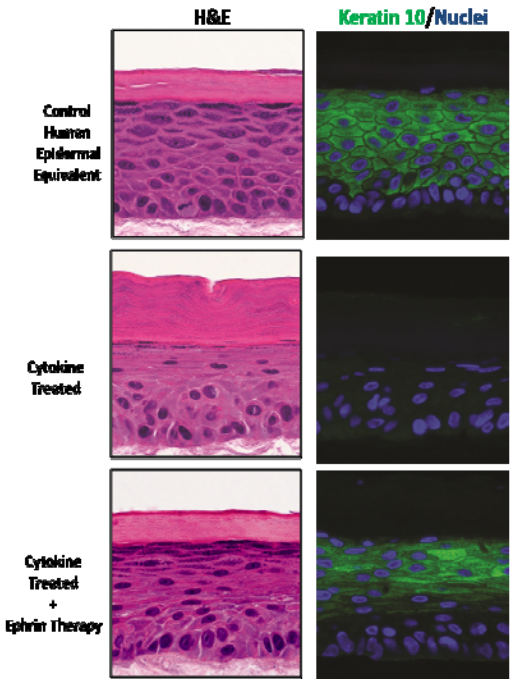
Amy S. Paller, M.D

An Eph-ective Way for Keratinocytes to deal with inflammation

by Spiro Getsios, SDRC Keratinocyte Core Director

The SDRC Keratinocyte Core specializes in the construction of 3-dimensional organotypic models of the human epidermis that allow researchers to study keratinocyte functions in a context that more closely reflects the situation in actual patient skin. Researchers throughout the United States have come to the Keratinocyte Core facility to train in the raft culture technique, which is so called because skin cells 'float' at an air-liquid interface to allow for their development into a stratified epithelium.

Raft cultures have been used many by our investigators for a number of purposes. For example, one can observe how a particular gene and protein expression changes over time as a stratified epithelium regenerates itself. One can also analyze changes in cellular and subcellular architecture during normal epithelial differentiation using a variety of imaging techniques. The 3D epidermal equivalents also provide an interesting complement to mouse models of human disease and these raft cultures only take 2 weeks to develop to maturity. Silencing a gene of interest or introducing a mutant protein into keratinocytes allows one to study its importance to human epithelial cell function in a relatively short time frame. It is also possible to use human patient cells to rebuild their epidermis and use as a model for their disease. These human skin equivalents can also be grafted onto immune-deficient mice for longer term studies.



Human epidermal equivalents were grown in the presence (middle panels) or absence (top panels) of pro-inflammatory cytokines to model how keratinocytes respond to inflammatory insults in psoriasis. Epidermal morphology (H&E staining; left panels) and keratinocyte differentiation (keratin 10 immunostaining; right panels) is impaired by cytokines. Treating cytokine-exposed epidermis with ephrins normalizes epidermal architecture and differentiation (bottom panels).

Recently, the Getsios lab used the 3D raft model to study how the epidermis deals with inflammation in a common skin disease, known as psoriasis. Patients with psoriasis have patches of skin that are thick, flaky and irritated; their keratinocytes also differentiate poorly in the face of this inflammation. In collaboration with Johann Gudjonsson's group at the University of Michigan, the Getsios lab found that a novel skin signaling pathway known as the Eph/ephrin axis is altered in psoriatic patient skin (Gordon et al., JID; 2012). Specifically, ephrin-A ligands that are important for activating the EphA2 receptor to trigger keratinocyte differentiation (Lin et al., MBC; 2010), are depleted by inflammatory cytokines in the psoriatic epidermis leading to an increase in the EphA2 receptor. The SDRC Keratinocyte Core helped model this inflamed state in keratinocytes by growing raft cultures in the presence of cytokines that are elevated in psoriasis. Using this 3-D model of human psoriatic epidermis, the Getsios lab was able to show that adding back ephrin-A ligands can normalize differentiation in keratinocytes dealing with inflammatory insults (Fig. 1). These studies suggest that topical delivery of EphA2/ephrin-A signaling axis regulators may be useful when used in combination with immune therapies to treat patients with skin inflammation.

References:

Samantha Lin, Kristin Gordon, and Spiro Getsios (2010). Ligand targeting of EphA2 enhances keratinocyte adhesion and differentiation via desmoglein 1. *Mol Biol Cell* 21:3902-14.

Kristin Gordon, James J Kochkodan, Hanz Blatt, Samantha Y Lin, Nihal Kaplan, Andrew Johnston, William R Swindell, Paul Hoover, Bethanee J Schlosser, James T Elder, Johann E Gudjonsson, and Spiro Getsios (2012). Alteration of the EphA2/Ephrin-A signaling axis in psoriatic epidermis. *J Invest Dermatol* (in press)

The Latest News from the DNA/RNA Core

by Alexander Yemelyanov, MD, PhD

1. Using viruses for infection of normal human primary fibroblasts growing in 3D collagen matrix.

Human and mouse fibroblasts are the major cell population in dermal compartment of the skin, and are essential for the generation of organotypic raft cultures (ORC, 3D skin cultures). Even though the infection of fibroblasts in 2D cultures is well-understood, it is still quite unknown if lentiviruses can infect cells growing inside a 3D matrix.

The goal of scientists from the laboratory of Dr. Ramille Shah (Materials Science and Engineering, Evanston campus) was to optimize the conditions for infecting normal human fibroblasts (NHF) growing in 3D collagen matrix. The series of experiments in Dr. Shah's lab demonstrated that lentivirus applied on the top of the collagen matrix was able to enter the matrix and retained its high infection capacity. More than 70% of primary fibroblasts were infected with GFP-expressing virus as shown in the Figure 1. Overall, the efficacy of infection was only 30% lower compared to the virus efficiency in equivalent 2D NHF cultures. To estimate the amount of the primary fibroblasts infected inside the 3D matrix, cells were isolated from the matrix by collagenases and analyzed using FACS (Figure 1.C).

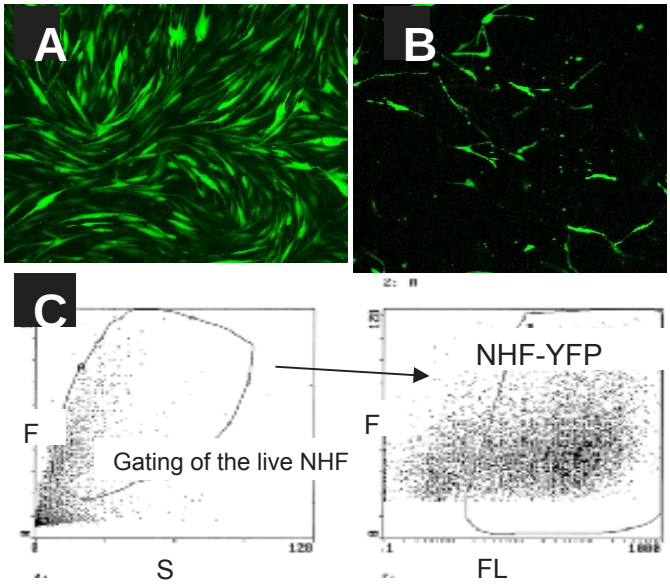


Figure 1. Infection of normal human fibroblasts (NHF) by GFP-expressing lentivirus in 2D cultures and 3D collagen matrix. A. Close-to -100% infection efficiency in 2D NHF culture. 200 x 10^3 NHF were plated onto 6 well plate and infected with 0.5 ml of the 10^7-10^8 TU/ml lentivirus for 6 -8 hours. The expression was visualized by day 5 with a Zeiss inverted fluorescent scope. **B.** Infection of NHF growing inside a 3D collagen matrix in a 12 well plate via application of lentivirus (0.5 ml, 10^7-10^8 TU/ml) on top of the collagen matrix. The expression was visualized by day 5 as in A. **C.** FACS analysis of GFP-expressing NHF growing in 3D collagen matrix. NHF infected as in **B** were isolated from the collagen matrix by collagenases.

These experiments suggest that the lentiviral particles can penetrate the 3D collagen lattices, diffuse within it, and infect the cells. This approach can be extended for infection of other cells types grown in 3D conditions in vitro in other models such as ORC (skin rafts), and other organic cultures. DNA/RNA Delivery Core and Keratinocyte Core are planning to test infection of NHEK growing in ORC. We expect that the virus may be able to reach the NHEK within the RAFTs by diffusion through collagen support when directly added to the keratinocyte culture media.

2. Successful application of the high titer viruses for in vivo injections into the mouse brain.

Although primary focus of the SDRC and DNA/RNA Delivery Core is on generating lenti- and retroviruses for keratinocyte and epithelial research, the Core lentiviral products are in demand by NU scientists working in other fields of Life Sciences. Currently, the Core generates high titer lentiviruses with titers up to 10^{10}-10^{11} TU/ml suitable for in vivo research. The high titer lentiviruses have been successfully used for injections into mouse and rat brain by NU researchers working in the fields of Neurology, Physiology and Behavioral science (Laboratories of Drs. Radulovic, Disterhoft, Chetkovic and Xu). Here we illustrate one of the latest successful applications of the Core lentiviruses for the intra-brain injections. These experiments were performed by Dr. Jian Xu (Department of Physiology) (Figure 2).

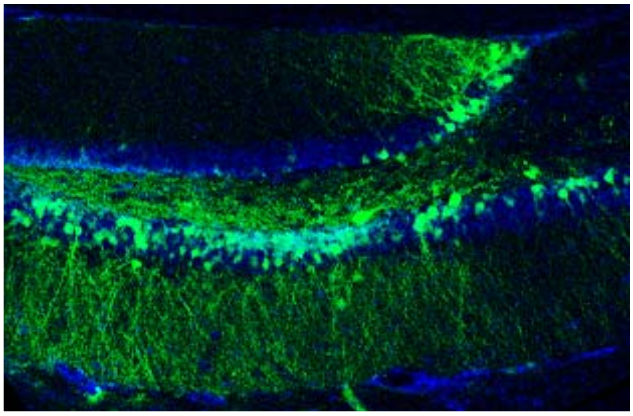


Figure 2. Lentiviral infection of the mouse hippocampus. GFP-expressing lentivirus (2 ul) was injected into mouse hippocampus. GFP expression was evaluated 14 days after the injection in whole hippocampus histological sections.

3. New vectors acquired and tested by the DNA/RNA Delivery Core.

3.1. Mammalian expression vector for simultaneous expression of multiple proteins. The DNA/RNADelivery Core acquired a pULTRA lentiviral vector (Addgene) that allows to express up to four proteins at the same time under the single promoter. In this vector the protein expression is driven by the native proximal PGK1 promoter that triggers expression of the proteins within one transcript. The proteins in the transcript are separated by self-cleaving short peptide links (T2A, P2A and F2A links) recognized by the ribosome during translation in mammalian cells. The latter allows for simultaneous expression of several proteins at the same molar amounts and in the same cell population.

3.2. New vectors for shRNA/miR expression

The Archival Tissue Bank: A Gateway to Translational Research

by Robert M. Lavker, PhD - SDRC Pathology Core co-Director

One of the unique features of the SDRC Pathology Core is our archival tissue bank (SDRC-ATB), which consists of paraffin embedded blocks of biopsies from "normal" human skin, as well as skin from patients with a variety of skin diseases (Table 1). For example, investigators interested in surveying whether the expression of their novel protein may be altered in diseases of abnormal keratinization have access to psoriatic epidermis, atopic dermatitis, porokeratosis or epidermolytic hyperkeratosis, to name a few (Figure 1). Alternatively, if one believes their protein of interest may be related to a neoplastic event, tissues from patients with basal cell carcinoma, squamous cell carcinoma, melanoma, as well as actinic keratosis can be surveyed. For those SDRC members interested in looking at hair follicle abnormalities, biopsies are available from patients with keratoacanthoma. Archival specimens of sun damaged skin are also available for investigators interested in the effects of UV radiation on the skin. Furthermore, archival specimens are available from subjects that were exposed to UVB vs UVA radiation for fixed times as well as 320nm,340nm, 360nm and 400nm irradiated sites. As one can see, the possibilities of going from the bench to the bedside are limited only by one's imagination.

Another aspect of the SDRC-ATB) is the access that SDRC members have to epithelial tissues other than skin. For example, the SDRC - ATB has blocks from "normal" human conjunctival, limbal and corneal epithelia for use in surveying protein expression. Samples of plantar and palmar epithelium are also available. Needless to say, any of the archival paraffin embedded specimens from normal and/or diseased skin can be used for laser capture microdissection (another Pathology Core service) if expression profiling is desired. The SDRC-ATB also has a limited supply of normal human skin that has been fixed specifically for in situ hybridization. Similarly, there are a limited number of specimens that are fresh frozen and embedded in OCT for immunohistochemistry.

It is relatively painless for SDRC members to acquire blocks from the SDRC-ATB. The process requires that investigators fill out a form (Figure 2). In order to obtain these forms, please contact the Tissue Acquisition Coordinator Natasha Thakkar at natasha.thakkar@northwestern.edu. The request is then sent to the committee for review and is approved once all concerns have been worked out. After approval a team works on acquiring the specified tissue requested.

Table 1. Archived Priority Diseases Available

Archived Tissue Available
Psoriasis
Spongiotic Dermatitis
Squamous cell carcinoma well differentiated
Squamous cell carcinoma moderately well differentiated/acantholytic type
Basal cell carcinoma, nodular type
Basal cell carcinoma, infiltrating type
Basal cell carcinoma, superficial type
Lichen planus
Epidermolytic hyperkeratosis
Seborrheic keratosis
Actinic keratosis
Verruca vulgaris
Normal non-sun damaged skin (excluding face and scalp)
Normal sun damaged skin (in face)
Porokeratosis
Morphea
Keratoacanthoma

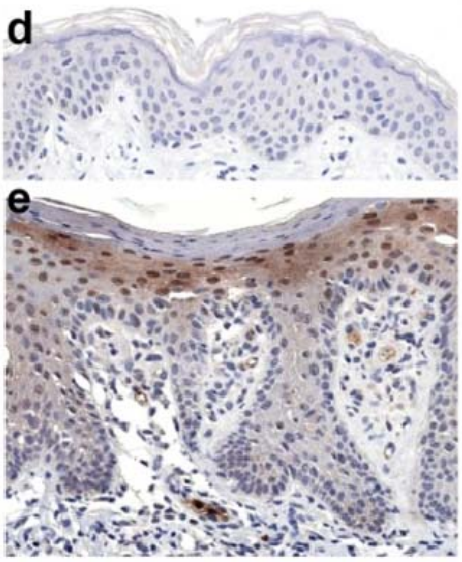


Figure 1. Factor inhibiting hypoxia –inducible factor-1 (FIH -1) expression is elevated in conditions of abnormal keratinization. Immunohistochemical localization of FIH-1 in paraffin-embedded specimens of normal (upper panel) epidermis and the epidermis from a patient with psoriasis (brown reaction product, lower panel). FIH-1, which negatively regulates Notch signaling, is markedly increased in the psoriatic epidermis compared with normal epidermis. Skin diseases such as psoriasis have been linked to abnormal Notch signaling and thus the elevated FIH-1 staining in psoriasis is evidence that FIH-1 expression is correlated with Notch activity and associated with impaired epidermal differentiation.

Getting to Know the SDRC featuring Greg Smith and Liang Zhou

Gregory A. Smith, PhD, Associate Professor Microbiology- Immunology

What is your hometown?

I was born in Elmhurst, IL. However, my family moved to southern California about a year later

What brought you to Chicago?

Northwestern. When looking for faculty positions, my wife (Margrit Urbanek, also a member of the Feinberg faculty) and I considered offers from several universities across the country. Dr. Patricia Spear was then chair of the Department of Microbiology-Immunology and asked me to consider a position in her department. The colleagues, resources, and environment of Northwestern were attractive to both of us, and Drs. Dunaif and Spear made compelling arguments for us to come.

What do you like to do on your spare time?

An occasional drink with my colleagues & relaxing and playing games with my family. Although I have little free time, I enjoy taking on home improvement and woodworking projects; however, the tools in my garage are getting quite dusty.

Where did you get your undergraduate degree? I got my B.A. in Microbiology from the Univ, of California: Santa Barbara.

What attracted you to Northwestern and the SDRC?

Although my training is in the basic research underlying bacterial and viral pathogens, my research program is both interdisciplinary and clinically relevant. The diversity of research was a large factor in coming to Northwestern. From a more personal level, I was attracted by the incredible location of the medical school. Few medical schools are situated in an attractive area of a large city. Being near the lake and Michigan Ave is incredible. My recent appointment to the SDRC started off as an interest in the viral vector core but the overlaps between dermatology and herpesvirus infections, from cold sores to shingles, are of larger significance. One of the SDRCs strengths is the bridge it builds between disciplines, which exemplifies one of the greater benefits of working at Northwestern.

What are your research interests?

Since graduate school I have been enthralled by how intracellular pathogens manipulate host cell biology. While disease caused by pathogens can in some cases be devastating, the tactics that these organisms have evolved to manipulate us are fascinating. Pathogens have a lot to teach us, and turning their resources to the development of useful molecular tools and, ironically, disease treatments is an exciting endeavor.

What exciting projects are you working on?

Everyone in my laboratory is doing research that I find very exciting, and seeing them overcome the challenges that are pervasive in research and making new discoveries is the best part of my work. Two of the big questions that we are focused on is how some herpesviruses invade the nervous system, and how herpesviruses assemble and infect cells (i.e. the mechanics of the viral nanoparticle). Unlike many "accidental" viral infectious of the nervous system (such as polio and the encephalitis that accompanies many arbovirus infections), some herpesviruses are exquisitely designed to deliver their genetic content to the nervous system. How do they do it? The process of neuroinvasion is poorly understood but we are making new discoveries that explains some of this unique biology. Recently, we have developed tools to examine the intra-viral architecture in unfixed, infectious, samples. We are now seeing details of the 200 nm herpesvirus structure that have been unresolved. This achievement is a fundamental advance in that we can apply these techniques to imaging infections of living cells and witness how viruses assemble, disassemble, and interact with cellular structures in unprecedented detail.

Where did you work prior to Northwestern? What type of research did you do there?

I received my Ph.D. from the University of Pennsylvania where I studied how the intracellular bacterial pathogen, Listeria monocytogenes, enters cells and subverts the mammalian cell cytoskeleton to move within and between cells. You owe it to yourself to watch a time lapse recording of Listeria propelling through cells if you've never seen it. Listeria essentially exploits the polymerization potential of the actin cytoskeleton and turns it into a motile force, and in this way transmits from cell to cell without leaving the intracellular niche. This tactic allows the bacteria to avoid the host humoral response. I then went to Princeton University to work with Dr. Lynn Enquist and study the neuroinvasive herpesviruses. Although researchers do not often switch between the study of bacterial and viral pathogens, it seemed like a natural transition to me. My interest remained on the cell biology underlying infection. It was during this time that I developed a genetic system for studying the herpesviruses and performed the first live-cell imaging of the herpesvirus particle in action as they raced from nerve endings, down the lengths of axons, and to the neuronal nucleus where they inject their DNA genomes through nuclear pores. Once this happens, you are infected for life and there is no cure.

Who/what has had the biggest influence in your life?

I have to name three. Dr. Julie Theriot, who I had the privilege of collaborating with during my graduate studies. Her brilliance was palpable and her childlike excitement at the wonders of biology: infectious. Dr. Lynn Enquist, my postdoctoral mentor, taught me more about research and science than any other one person. To this day, he remains my role model. And most notably, my father, who is a constant positive influence and source of encouragement throughout my life.

Liang Zhou, MD, PhD, Associate Professor Pathology, Microbiology, and Immunology

What is your hometown?
Nanjing, China

What brought you to Chicago?
Nice city and good scientific environment

What do you like to do on your spare time?
Spending time with my newly born daughter

Where did you get you undergraduate degree?
Nanjing Medical University

What attracted you to Northwestern and the SDRC?
The most attractive feature is the collegiality among faculty members and the nurturing scientific environment. As a junior faculty member, mentorship is critical for career development. I appreciate the guidance and advice that I have received from my colleagues since I started my own lab two years ago. I hold a joint appointment with the Department of Pathology and the Department of Microbiology and Immunology. Both departments (e.g., my chairmen and colleagues) support me 100 percent without reservation. I am also impressed by the professionalism of the administrative staff in my home departments, who have made my daily life as a PI easier and more enjoyable.

What are your research interests?
My most significant research contribution has been the discovery of the master regulator of T helper (Th)17 cells called RORγt. We demonstrated that Th17 cells represent a novel T cell subset that is implicated in many human illnesses (e.g., infections, autoimmune diseases, and cancer). Since establishing my independent laboratory at Northwestern University, I have continued to characterize the molecular interactions among transcription factors that govern the development of Th17 and other RORγt immune cells (e.g., innate lymphoid cells, or ILCs).

What exciting projects are you working on?
The crosstalk between the environment and host immune system

Where did you work prior to Northwestern? What type of research did you do there?
New York University. Basic immunology research to understand T cell development in health and disease

Who/what has had the biggest influence in your life?
My late parents. My father was a biochemist and my mother was a pediatrician specialized in Hematology/Oncology. They encouraged me to be a scientist and to pursue what inspire me the most: Make new discoveries.

Mark Your Calendars!

Epithelial Biology Seminar Series

Ward #3-015 from 12Noon - 1:00pm

May 2, 2013 Presenter: Troyanovsky Lab of Dermatology
May 30, 2013 Presenter: Xiao-qi Wang, MD, PhD of Dermatology

In conjunction with Tumor Invasion, Metastasis and Angiogenesis (TIMA), NU-SDRC sponsors this multidisciplinary program which provides a forum for laboratories to present their research in a "work in progress" format to obtain feedback from a large group with wide ranging of expertise. Currently participating laboratories come from a variety of departments, including (although not limited to) Cell and Molecular Biology, Dermatology, Medicine, Microbiology-Immunology, Pathology, Pediatrics, Urology and Surgery. Areas of interest are diverse; selected research programs that are regularly represented focus on intermediate filament biology, adhesion and cell motility, stem cell biology, signaling, wound healing, and host-pathogen interactions.

For additional information visit The Epithelial Cell Biology website listing: **http://www.cmb.northwestern.edu/episeminars.htm** or contact Betsy Cutcher at **e-cutcher@northwestern.edu**.

NU-SDRC Research Retreat

The **2013 Retreat will take place on Tuesday, June 4th, 2013** from 8:30-11:30am in the Baldwin Auditorium followed by a poster session in the Ryan Auditorium until 1:00pm at the Robert H. Lurie Medial Research Center at 303 E. Superior. For additional information contact Betsy Cutcher at e-cutcher@northwestern.edu.