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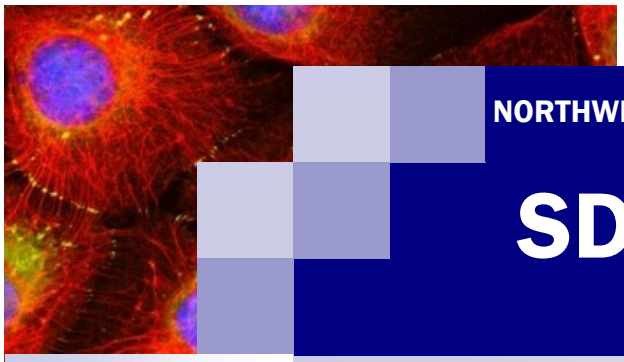


NORTHWESTERN UNIVERSITY SKIN DISEASE RESEARCH CENTER

SDRC NEWSLETTER

Inside this issue:

WELCOME!

As Principal Investigators of the Northwestern University Skin Disease Research Center (NU-SDRC), we are delighted to introduce you to the exciting progress in mucocutaneous biology research at our Institution. The NU-SDRC was established in the summer of 2009 as one of 6 NIH-funded sites with Cores for investigative research in skin disease. We strongly encourage you to visit our website, http:/skinresearch.northwestern.edu, for a real-time update of services and exciting new developments. All Center Cores are housed in a dedicated facility on the 13th floor of the Ward building.

We are adding this newsletter as a way to regularly disseminate information of interest to the Northwestern community. If you are already a member of the SDRC, our goal is to insure that you are up-to-date on progress being made at Northwestern in Epithelial Biology that utilizes our Cores. If you are not currently doing research related to mucocutaneous biology, we encourage you to take a look at the services offered by the Cores that may be of value to your research and to consider collaborating with a SDRC member or transitioning your research to skin disease. We've just opened our annual competition for SDRC **Pilot and Feasibility grants**. If you are not currently a member of the SDRC, consider submitting a 2 page application for a chance to obtain these startup funds, as well as huge discounts on Core services. Being an SDRC member promotes collegiality and collaboration – and enables access to unique Core services at very affordable prices.

As you read this inaugural edition of the SDRC newsletter, we hope you will share our excitement that so many investigators from 11 departments within the University are already using the SDRC's Cores to support their research. Our **Keratinocyte Core** provides cultured keratinocytes, including 3-D skin equivalents (organotypic raft cultures) that simulate full-thickness epidermis. For investigators with a transgenic or knockout mouse model, the Core can establish primary or immortalized mouse keratinocytes for specific epidermal cell studies. To supplement the capabilities of the Keratinocyte Core, the SDRC was jointly awarded with the NU Imaging Core facilities a Nikon BioStation live imaging system, perfect for studying the movement and membrane-based activity of cells in real-time.

Our **Pathology Core** specializes in the pathologic assessment of skin, hair and nails, whether in mouse models or in cultured skin equivalents. The expertise of Core staff in dermatopathology makes use of this Core perfect for any investigator who has a mouse with a skin phenotype. The Pathology Core also enables researchers to use laser capture microdissection to isolate cells within tissue and a Franz cell apparatus for measuring transepidermal flux of potential therapeutic agents, now with radiolabeling capability.

Gene modification in keratinocytes has been difficult and largely depends on the introduction of viral vectors. The **DNA/RNA Delivery Core** has developed highly innovative and affordable tools for infecting epithelial cells with lenti- and retroviral vectors. Stay tuned in that Core for the arrival soon of inducible and suppressible transgene expression.

Finally, although not formally a Core within the SDRC, the Dermatology Department hosts a **Translational Core** that facilitates the acquisition of material from patients with skin disease (or controls) for SDRC members and can counsel about the transition of bench research to the clinical arena for Phase I trials. If you are considering applying your research to human skin disease, we would be glad to provide consultation and facilitation.

We wish all of you a very happy New Year.

Amy Paller, MD and Robert Lavker, PhD
Principal and Co-principal Investigators, SDRC

Save the Date for these Upcoming Events!

Epithelial Biology Series will be held on **Thursday, February 17, 2011** from 12Noon-1:00pm in Ward 4-075 and will feature Michael Werner, PhD from the Brian Mitchell Lab in Cell and Molecular Biology.

Lecture in Life Sciences Series featuring Dennis Roop, PhD, University of Colorado discussing his work with differentiation of induced pluripotent stem cells into keratinocytes on **Tuesday, March 8, 2011** at 4:00pm in the Lurie Hughes Auditorium.

The Keratinocyte Core Grows on You

by Paul Hoover, Spiro Getsios, PhD and Kathleen Green, PhD

The Keratinocyte Core provides a reliable source of cultured human epidermal keratinocytes isolated from newborns and adults to support studies in epithelial biology (see citations listed in Acknowledgement article). For example, the Getsios lab has recently taken advantage of these primary cultures to identify a key ligand-receptor (ephrin-EphA2) system that promotes keratinocyte differentiation (Lin et al., 2010). These studies will serve as a platform to determine whether the EphA2 receptor can serve as a therapeutic target in skin diseases where the differentiation program is impaired, such as psoriasis, ichthyosis and eczema. Work that was once limited to cell lines can readily be extended to these more native primary cell cultures and is facilitated by state-of-the-art gene delivery and silencing techniques offered through the DNA/RNA Delivery Core.

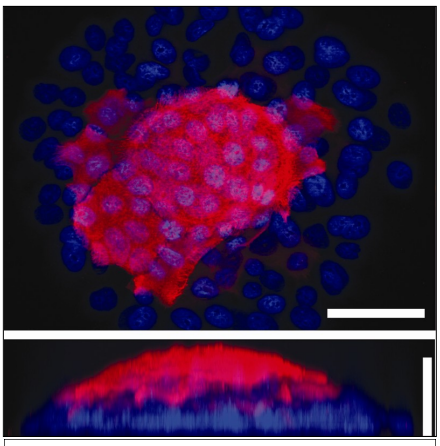


Image (top- X-Y plane; bottom- X-Z plane) depicting primary human epidermal keratinocytes forming stratified piles in culture following ephrin ligand activation of EphA2 (Lin et al. 2010). Keratin 10 is stained in red and is restricted to the more differentiated, stratified cells. DAPI stains the nuclei in blue. Top Bar = 50 mm; Bottom Bar = 10 mm.

In addition to epidermal cells obtained from normal skin, the Core is initiating and immortalizing keratinocyte cultures from consented patients with skin diseases. Drs. Kathy Green and Amy Paller collaboratively utilized this service to grow cells from a baby with a lethal skin blistering disease (lethal acantholytic epidermolysis bullosa) and gain further insight into the pathomechanisms that lead to this fatal disease (Hobbs et al., 2010). Partnership with the Translational Core Tissue Acquisition services makes it possible to work with clinically relevant experimental cell cultures and allows investigators to test novel therapies for skin disease.

Wouldn't it be convenient to have a keratinocyte cell line of your knock-out or transgenic mouse model with a skin phenotype? Let us help you dissect a complex molecular pathway in a cell culture system instead of a complicated living animal. Rob Hamaka and the Chandel lab think so and have utilized our Core's new service of isolating and immortalizing murine epidermal keratinocytes using their model of skin-specific mitochondrial deficiency.

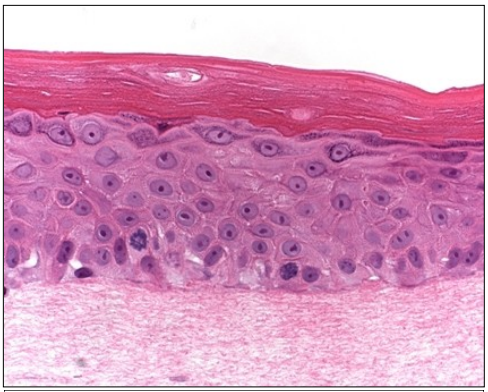
Finally, we are moving keratinocyte cultures into the 3rd dimension thanks to our consultant, Dr. Laimins. Along with Drs. Green and Getsios, organotypic raft models of human epidermis permit the analysis of epithelial morphogenesis in a culture model that accurately reflects skin disease, such as bacterial induced blistering (Simpson et al., 2010). Histological and immunohistochemical analysis of these 'raft' cultures, established from normal or virally transduced keratinocytes, is simplified by our close relationship with the Pathology Core. We are currently adapting this model for live cell imaging, wound healing, drug delivery and viral transmission studies, and we will soon be offering the services of grafting these tissues onto the backs of immunocompromised mice for long-term tissue homeostasis studies.

To find out more about our services and opportunities, please contact Paul Hoover at paul-hoover@northwestern.edu.

Pathology Core-a Cut Above the Rest

by Hanz Blatt and Robert Lavker, PhD

Does your mouse model have a skin phenotype? The Skin Pathology Core can answer that question if you're not sure, and is the ideal site for your pathology needs if you are doing skin research. Our most basic and highly demanded services are processing and interpretation of sections from paraffin-embedded and cryopreserved epithelial tissues. In our first year, we processed more than 2,700 samples for investigators at steeply discounted prices. Among these were sections that helped Drs. Doug Vaughan and Mesut Eren from the Department of Medicine, Cardiology Division to elucidate the reason that plasminogen activator inhibitor-type I (PAI-1) overexpression leads to a hairless mouse phenotype. Similarly, Nav Chandel from the Department of Medicine, Division of Pulmonary and Critical Care found that knocking out mitochondrial ROS in epidermis specifically led to alterations in hair and sebaceous gland development. These mice had irregular hair follicles, which appeared to be in the catagen (destructive) phase of the hair growth cycle compared with the wild type mice whose hair follicles were regular and in the anagen (growing) phase of the hair growth cycle. Furthermore, hair follicles of the mice lacking mitochondrial ROS did not have sebaceous glands, whereas wild type mice had fully developed sebaceous glands. Dr. Tom Mustoe from the Department of Surgery used the Core to assess the influence of epithelial-connective tissue interactions on scar formation. We also provide the service of sectioning the very delicate 3-D skin equivalents (see Keratinocyte Core article). As an example, the Mirkin laboratory from the Chemistry Department on the Evanston campus discovered that his fluorophore-labelled siRNA-gold nanoparticles are able to traverse the mouse epidermal barrier through our Pathology Core services and then confirmed the ability of these siRNA-gold nanoparticles to penetrate human epidermis through the use of 3-D skin equivalent sections processed in our Core, as well as with our Franz cell apparatus (Continued on page 3).



An example of an H&E section (Pathology Core) of an organotypic raft culture (Keratinocyte Core) generated from primary human epidermal keratinocytes retrovirally transduced with FIH-1 (factor-inhibiting hypoxia-inducible factor 1).

Put Your Left Gene In, Take Your Right Gene Out, Let the DNA/RNA Delivery Core Shake Your Keratinocytes All About

by Irina Budunova, PhD, MD & Alexander Yemelyanov, PhD, MD

The objective of DNA/RNA Delivery Core is to provide SDRC researchers and epithelial biologists at Northwestern University with highly innovative tools for gene modifications. During the last year, the Core has made great strides in developing services to support the entire University community in gene introduction, particularly through lentiviral systems, to overexpress or knock-down genes.

- We now have extensive experience in infecting several human primary cells with lenti- and retroviruses, among them epidermal and corneal keratinocytes, prostate and urothelial cells, and endothelial cells.
- Lentiviral stocks generated by our Core have ~10^7 – 10^8 TU (transfection units)/ml titers, similar or exceeding those of commercial stocks, but at a fraction of the cost.
- In addition to standard lentiviral cassettes with different tags for cloning of cDNAs and shRNAs of your choice, we now offer lentiviruses expressing luciferase reporters for transcription factors such as AP1, NF-kappaB, and p53. These luciferase reporters also express YFP, allowing evaluation of transcription factor activity at the single cell level using fluorescence microscopy or FACS. Our lentiviruses expressing CMV.Cre recombinase facilitate deletion of floxed genes, such as in primary cell cultures from floxP mice. **Soon to come**: lentiviral expression vectors that allow controlled transgene expression.
- Our lentiviral packaging systems are fully compatible with libraries of ORF/cDNA, shRNA, and MiR pre-cloned in lentiviral expressing vectors that are available from major commercial sources, such as Open Biosystems, GeneCopoeia, System Biosciences and Addgene. Simply buy the expression vectors with inserts of your choice, and we will make lentivirus for you! With the permission of researchers, we are building an archive of these lentiviruses expressing cDNAs, shRNAs, and luciferase reporters. Feel free to contact us directly at a-yemelyanov@northwestern.edu. We may already have what you need for your research!

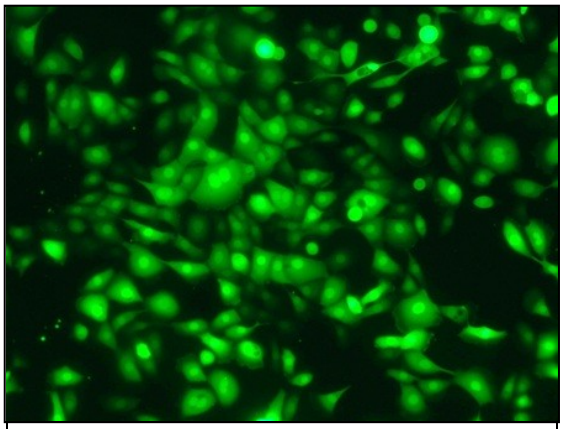


Image of the GFP expressing normal human keratinocytes.

Pathology Core (continued from page 2)

Our relationship with the clinical Dermatopathology Division at NMFF, also directed by the Pathology Core director, Dr. Joan Guitart, allows affordable access to the highest quality *in vitro* diagnostic (IVD) tools for bench research, and is a huge incentive for SDRC members. We have developed an array of skin-disease specific antibody stains for use in our DAKO Automated Immunohistochemical (IHC) Staining System that provides NU researchers with reproducible high-throughput staining with a quick turnaround time.

This state-of-the-art system allows the simultaneous staining of 24 antibodies across 48 slides in a single experiment without the worry of systematic variation. We also boast Envision's Dual Link Secondary System, which reduces virtually all non-specific secondary binding in tissues. All slides are precipitated with DAB or AP substrates for permanent visualization. As in our routine histology service, our antibodies undergo the same rigorous validation protocol used for IVD at NMFF for patient diagnosis. To date, we have used automated IHC for detecting BrdU, Ki67, Keratins 3, 5, 14, and 10, as well as differentiation markers such as involucrin, filaggrin, loricrin, desmogleins 1 and 2, and CD34.

In addition to immunohistochemical staining, we also provide immunofluorescence (IF) analysis, and will tailor an IF protocol to fit the specific researcher's need. This includes our "New Antibody Evaluation" service, in which we purchase an antibody of your choice and subsequently work out staining dilutions, fixations, and protocols for a nominal fee. This is a great option for those high-risk/low-reward antibodies that are bound to soak up your time and money. The Core retains the antibody to provide continuing discounted service for you and potential collaborators at the Institution.

Our Pathology Core does more than just cut and stain sections. Our laser capture microdissection system enables the precise isolation of epithelial tissues of interest. For example, it is possible to isolate basal cells from the more superficial cells and extract the RNA for expression profiling. We have found that expression profiling can accurately be performed with as few as 300 cells. The laser capture microdissection apparatus is ideal for isolating various portions of a tumor for subsequent analysis. Our system enables fresh frozen tissue to be used with a minimum of staining and fixation, thus yielding high quality RNA. We also have a Franz cell apparatus that allows the assessment of flux of a potential therapeutic agent or pathogen through epidermis. Dr. Thomas Hope utilizes abdominal tissue samples from the surgical theater for use in *in vitro* explant studies to investigate the rate at which the HIV retrovirus can penetrated a diverse range of epithelial tissues. Similarly, Dr. Dinh utilizes the tissue processing service in conjunction with our Franz cell apparatus to investigate the diffusion rate of tritiated water through penile and cervical epithelia. By working with the Pathology Core, these two primary investigators have furthered the understanding of HIV transmission.

We invite you to contact us (h-blatt@northwestern.edu) or stop by our laboratory at Ward 13-049 to find new and exciting opportunities for your research needs.

Meet the SDRC featuring Paul Hoover

by Betsy Cutcher and Paul Hoover

Paul Hoover is the Keratinocyte Core Research Technologist and has been instrumental in the establishment and growth of the Core. Let's learn a bit more about Paul....

So Paul, where is your hometown?
I am originally from Gleed, Washington, which is a very small town outside Yakima. I have spent most of my life in San Antonio, TX and really consider that to be my hometown.

What brought you to Chicago?
My wife wanted to be closer to her family and her job, so we decided to make the move.

What do you like to do in your spare time?
My wife and I have very recently had a baby, so I don't believe spare time exists anymore. When I do get a few minutes these days, I enjoy the quiet...and a little television.

Where did you obtain your undergraduate degree?
I studied at the University of Texas at San Antonio and earned a BS in Biology with a minor in Chemistry.

What attracted you to Northwestern and the Skin Disease Research Center?
What mostly attracted me was a new opportunity with a prestigious University, along with the chance to work in a field different from my previous work. I really wanted to branch out and try something new. I also was drawn to the idea of being able to help PIs work on their individual projects because every day brings something different.

What are your research interests?
I am relatively new to this field and exploring epithelial biology in many aspects. Due to my previous research focus on mitochondria distress and ROS in kidney disease I am currently intrigued by Dr. Navdeep Chandel's research involving mitochondria distress and ROS and hope to explore this area of research further.

What exciting projects are you working on?
The newest project involves working with Dr. Kathy Green's lab in Pathology in setting up raft to graft experiments. Raft cultures will be set up using de-epidermalized dermis and grafted onto SCID mice.

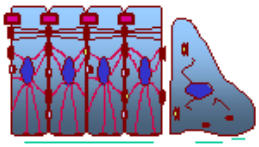
What has been your best experience at Northwestern thus far?
The people; everyone has been very helpful in explaining the science behind their projects and, remarkably, do it with a smile.

The Epithelial Biology Series – More than Skin Deep

In conjunction with Tumor Invasion, Metastasis and Angiogenesis (TIMA), the SDRC sponsors this multidisciplinary didactic 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 broad 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. Epithelial Biology meets every other Thursday from 12:00-1:00pm in the Montgomery Ward Building, 303 E. Chicago Avenue, Room #4-075. Stop by one of our upcoming presentations:

February 17, 2011 Michael Werner, PhD from the Brian Mitchell Lab in Cell and Molecular Biology
March 3, 2011 Sergey Troyanovsky Lab from Dermatology (speaker TBD)
March 17, 2011 Samantha Lin from the Spiro Getsios Lab in Dermatology
April 7, 2011 Jeffrey Myers, MD, PhD, FACS from the University of Texas MD Anderson Cancer Center.

For additional information on upcoming presentations contact Betsy Cutcher at e-cutcher@northwestern.edu.



WHAT'S NEW AT THE SDRC?

SDRC and Cell Imaging Core Facility Awarded Joint Grant for New Nikon BioStation

The SDRC Administrative Core is proud to announce the recent award of a live cell imaging system for studying keratinocytes through a Small Instrumentation Grant from the NU Office for Research Shared and Core Facilities. The Nikon BioStation will be housed in the NU Core Imaging Facility on the second floor of the Searle and Morton Buildings, providing SDRC members with both priority use and on-site expertise of the Core Imaging faculty (particularly Dr. Leong Chew) and staff. The Nikon BioStation IM-Q Live Cell Recorder is a novel, all-in-one microscope specifically tailored for live-cell incubation with stable CO_2 delivery, monitoring and long-term time-lapse imaging. The system allows for various observation methods in time, Z-step and has the ability to sequentially capture green and red fluorescence along with phase contrast. This new system will be of particular benefit to SDRC users studying keratinocyte motility and/or membrane molecular interactions. The Cell Imaging Core has a dedicated incubator on site to support fragile primary keratinocytes and allow rapid transfer to the microscope.

We are excited to integrate this new imaging system into our Core facility and will provide additional details on our website regarding its use in the upcoming weeks. For specific questions regarding the BioStation, contact the Cell Imaging Core facility at 312-503-2841 or the Keratinocyte Core at 312-503-4192.

How to Acknowledge the SDRC

Now nearly halfway through our second year, the SDRC Core facilities have continued to expand in services and users. As we continue to grow and support your epithelial research, please don't forget to properly acknowledge the SDRC in your research in scholarly reports, presentations, posters, and published materials. This acknowledgment provides a visible measure of the impact of the SDRC Core facilities and is essential for our continued growth and funding. We would also like to celebrate the successful research of our users and encourage collaborations by listing SDRC-acknowledged research on our website. We request that you forward your publication citation or a copy of any publications to display on our website to Betsy Cutcher at e-cutcher@northwestern.edu.

When you have a moment, check out the following publications of users supported by the SDRC Core facility.

- Hobbs RP, Han SY, van der Zwaag PA, Bolling MC, Jongbloed JD, Jonkman MF, et al. Insights from a desmoplakin mutation identified in lethal acantholytic epidermolysis bullosa. J Invest Dermatol. 2010;130(11):2680-3.

- Lin S, Gordon K, Kaplan N, Getsios S. Ligand targeting of EphA2 enhances keratinocyte adhesion and differentiation via desmoglein 1. Mol Biol Cell. 2010;21(22):3902-14. PMCID: 2982116.

- Simpson CL, Kojima S, Cooper-Whitehair V, Getsios S, Green KJ. Plakoglobin rescues adhesive defects induced by ectodomain truncation of the desmosomal cadherin desmoglein 1: implications for exfoliative toxin-mediated skin blistering. Am J Pathol. 2010;177(6):2921-37. PMCID: 2993287.

- Yu J, Peng H, Ruan Q, Fatima A, Getsios S, Lavker RM. MicroRNA -205 promotes keratinocyte migration via the lipid phosphatase SHIP2. FASEB J. 2010;24(10):3950-9.PMCID: 2996908.

When acknowledging the NU-SDRC in your publications, please use the following, NIH-required language:"This research was supported in part by resources provided by the Northwestern University Skin Disease Research Center (5P30AR057216-02), Chicago, IL with support from the NIH/NIAMS. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Northwestern University Skin Disease Research Center or the NIH/NIAMS."

Call for SDRC Pilot & Feasibility Grant Proposals

We are soliciting **Pilot and Feasibility** grant funding proposals from: a) established, federally funded investigators with no previous work in skin biology who may apply their expertise to a skin disease-related problem; and b) junior faculty members who choose to investigate some novel aspect of keratinocyte biology. Investigators from outside of the Dermatology Department are encouraged to apply. The ultimate goal is that these SDRC funded studies will lead to new RO1 skin-related proposals to the NIH. The Pilot and Feasibility studies are funded at a level of $25,000/year for a 2 year maximum. In addition to the annual award grant, recipients are given an additional 50% off of the already discounted fees charged to Core members. Our current studies supported by this program include:

1. Navdeep S. Chandel, PhD, Associate Professor of Medicine - Keratinocyte Mitochondria as Systemic Oxygen Sensors

2. Jaime Garcia-Añoveros, PhD, Assistant Professor in the Department of Anesthesiology - The Role of Keratinocytes as Independent Thermosensors

3. Douglas A. Kuperman, PhD, Assistant Professor in the Department of Medicine, Division of Allergy-Immunology - The Effect of IL-4 Receptor Signaling on Inflammation and Skin Barrier Function in Atopic Dermatitis

4. Chad A. Mirkin, PhD, Professor of Chemistry, Medicine and Material Sciences and Engineering and Director of the Institute of Nanotechnology - Nanoparticle Delivery of Oligonucleotides Targeting Missense Mutations in Keratinocytes

The format of the applications should be a **2 page document** describing the nature of the pilot and feasibility project, together with a CV of the PI and a list of his/her current funding. Applications should be submitted to PI, Amy Paller, MD (apaller@northwestern.edu) or co-I Robert Lavker, PhD (r-lavker@northwestern.edu) via **email no later than Tuesday, March 15, 2011**. The projects will be evaluated by our Pilot and Feasibility Committee and funding decisions will be made by Friday, April 15, 2011. The start date for these awards will be July 1, 2010.