

Northwestern University Skin Disease Research Center

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PI Message

As the Skin Disease Research Center enters its third year of providing service to scientists at Northwestern University, we are proud to report the success of our first group of Pilot and Feasibility study recipients in obtaining funding (3 of 4 recipients) to continue studies, as well as many new initiatives now available. We encourage all scientists at Northwestern to consider how their favorite molecule, pathway or materials might be applicable to studies in epithelial cells. In addition, if a disease model shows a phenotype that seems to affect the skin, hair or nails, please consider chatting with one of our investigators about further study and potential applicability to human disease. Investigators with federal funding are eligible to become members if working in skin disease – and then will receive the benefits of being part of the SDRC.

What are some of these benefits? We now have a new live imaging system run jointly by the SDRC and the Imaging Core facility that is perfect for studying the movements of keratinocytes. In addition, 3-D models of wound healing in raft cultures have been developed and validated by our **Keratinocyte Core**. You might also take advantage of techniques that allow immortalization of keratinocytes for propagation

and increased survival, but are reversible. The **Pathology Core** has perfected its automated immunohistochemical staining abilities – and can run tests on large numbers of samples rapidly using a growing panel of antibodies (visit www.skinresearch.northwestern.edu for the listing). And don't forget that we can help you isolate designated areas in tissue samples for RNA and other assessments with the use of the Pathology Core's laser capture microdissection system. Our **DNA/RNA Delivery Core** has now worked with investigators from 10 different departments to develop lentiviral or retroviral constructs and infect any cell of your choice – including several primary cells and cell lines. Given that the technology is so unique within the university, we have made the service available to any investigator and for any cell, even if the studies have nothing to do with epithelial or other skin cells. Finally, in the interest of helping to drive translational research, the Department of Dermatology's **Translational Core** has kindly agreed to work with the Pathology Core of the SDRC in developing a bank of skin tissues for the use of Northwestern scientists; we already have a large array of paraffinized tissues and are accruing frozen and fresh tissue materials. For more information about the tissue acquisition application process please contact our Pathology Core Technician, Sunny Yang at shuangni.yang@northwestern.edu.

Amy S. Paller, MD

To view a full copy of the newsletter, visit our website: <http://skinresearch.northwestern.edu/>



We've got your Surface Covered – the Keratinocyte Core

by Spiro Getsios, PhD and Paul Hoover

The Keratinocyte Core provides a reliable source of cultured human and mouse epidermal keratinocytes isolated from newborns and adults to support studies in epithelial biology. Our members have taken advantage of these primary cultures to help understand potential therapeutic targets for skin disease. Work that once was limited to cell lines can now be extended to more native primary cell cultures.

In addition to epidermal cells obtained from normal skin, the Core is initiating and immortalizing keratinocytes from consented patients with a variety of skin diseases (e.g. psoriasis, epidermolytic ichthyosis, Darier disease). To maintain a long-term supply of these limited specimens, immortalization of keratinocyte cultures is achieved using two strategies: 1) traditional introduction of human papilloma viral elements; or 2) pharmacological inhibition of Rho Kinase, which allows these cells to complete the differentiation process in 3-D organotypic models of the human epidermis known as raft cultures. Recently the Core, in collaboration with the Live Cell Imaging Facility, has procured a Nikon Biostation for live-cell imaging. This biostation allows investigators to examine the sealing of linear scratch wounds in real-time. The Core has also adapted wound healing studies for raft cultures of human epidermis to more accurately reflect tissue regeneration in this stratified epithelium. Dr. Jonathan Jones's laboratory (Cell and Molecular Biology) has incorporated these models in their wound healing studies.




Mark your Calendars!

2012 NU-SDRC 3rd Annual Research Retreat

The NU-SDRC will hold its third annual one-day Research Retreat on **Tuesday, June 12, 2012**. The Retreat is attended by faculty and staff from across the University with the goal of enhancing interactions among all NU-SDRC investigators. The Retreat will feature presentations discussing the research and services offered by the Cores, an update on the final progress of the P&F grants, abstract presentations and an epithelial biology-based poster session. Our 2012 Retreat will be held on Tuesday, June 12, 2012 from 8:30-11:30 am in the Baldwin Auditorium followed by a poster session in the Ryan Atrium until 1:00pm at the Robert H. Lurie Medical Research Center at 303 E. Superior. For additional information contact Betsy Cutcher at e-cutcher@northwestern.edu.

Epithelial Cell Biology Research Group

The SDRC in conjunction with Tumor Invasion, Metastasis and Angiogenesis (TIMA) 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 range of expertise. Presentations focus on intermediate filament biology, adhesion and cell mobility, stem cell biology, signaling, wound healing, and host-pathogen interactions. Join us for our next EBRI on **Thursday, March 8, 2012 from 12Noon - 1:00pm in Ward 3-015 featuring William J. Muller, PhD presenting "Early antiviral responses after herpes simplex virus infection."** For more information contact Betsy Cutcher at e-cutcher@northwestern.edu.



Capturing the Moment with the Pathology Core

by Robert Lavker, PhD

The Pathology core has been working to refine and improve our ability to isolate and capture unperturbed epithelial cells with our P.A.L.M. laser capture microdissection (LCM) unit. This summer, users have successfully isolated and captured relatively pure populations of limbal and corneal epithelial cells from post-natal eyes of 3-, 7- and 14-day old mice. During these developmental times, the limbal epithelium is a relatively small, one-two cell layer sheet, closely opposed to the conjunctival epithelium. Likewise, the corneal epithelium is often closely opposed to the eyelid epithelium and mucocutaneous junctional epithelium. Despite these challenges, Julia Katsnelson, a 2nd medical student at Rush University successfully isolated, captured limbal and corneal epithelium at these time points and obtained high quality RNA from these two distinct cell populations. The purity of these populations was confirmed by the detection of limbal-preferred genes in the RNA extracted from the isolated limbal epithelium and corneal-preferred genes in the RNA from isolated corneal epithelium. Sufficient RNA was obtained for future microRNA expression profiling.

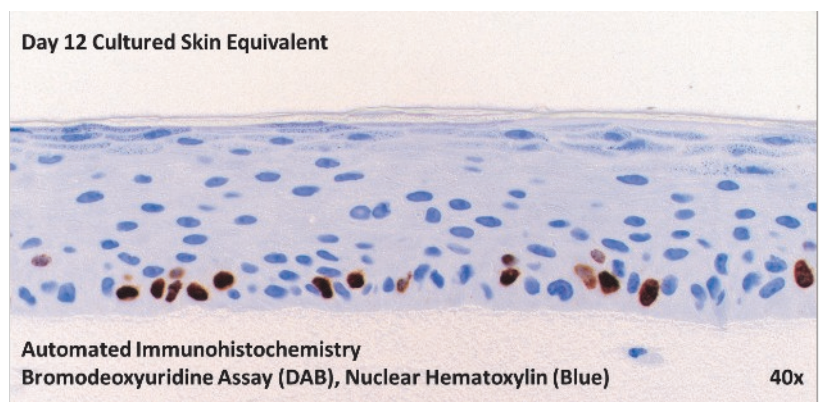
It is doubtful that most SDRC users will have such a challenging tissue isolation/capture project. Just think how easy it will be for you to isolate your favorite epithelium and/or epithelial cell-type from fresh frozen OCT-embedded tissues! The P.A.L.M. LCM unit will give you extraordinary yields of high quality RNA for expression profiling of real-time PCR analysis of cells and tissue in their "resting" state. To take advantage of this opportunity to capture cells and tissues contact Sunny Yang or Robert Lavker and make an appointment for a consultation.

DAKO Plus Automated Staining System: Available in the Pathology Core

by Hanz Blatt

The Skin Disease Research Center offers high-throughput staining via the Pathology Core. With access to an array of differentiation, proliferation, and cell junction markers, researchers can pursue the automated staining of up to 48 samples in a single run. Our system provides accurate and reproducible immunohistochemistry on paraffin embedded tissues. Our antibodies go through the same rigorous validation protocol that is used for in vitro diagnostic (clinical) IHC in the

Dermato-pathology lab at Northwestern. The secondary antibodies, DAKO Envision's Dual Link Secondary System, offer superior clarity for downstream analysis by removing the problem of non-specific secondary binding in tissues. For visualization, we use DAB or AP chromogenic precipitation and nuclear hematoxylin counterstains. Contact us today at r-lavker@northwestern.edu for additional information on these and other services offered by the Pathology Core.



DNA/RNA Delivery Core – An Infectious Team

by Alexander Yemelyanov, PhD

The objective of DNA/RNA Delivery Core is to provide SDRC researchers and epithelial biologists at Northwestern University with highly innovative viral tools for gene modifications. During the last year, the Core has significantly increased the titer of its lentiviral products, and these products can now be used on human and mouse primary cells and cell lines, either adherent or non-adherent. Currently, the DNA/RNA Delivery Core generates cost effective lentivirus and retrovirus with the titer of 10^8 - 10^9 TU/ml, of which 0.5 ml is sufficient to infect 200 - 300×10^3 cells plated in a well of the 6 well plate with close-to-100% efficiency.

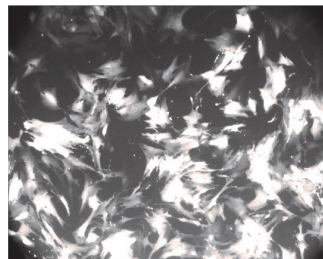
During the last year, the DNA/RNA Delivery Core has continued to improve viral technologies for protein expression and silencing. We specifically focused on:

- Development of protocols for cloning into standard viral expression vectors offered by the Core
- Protocols for lentiviral infection of primary normal human keratinocytes that can be used in *in vitro* biological NHEK models, such as organotypic raft cultures and NHEK colony assays
- Integration of Core products and services into the activities of other NU core facilities
- Development of protocols for infection of non-keratinocyte human and mouse primary cells and transformed cell lines

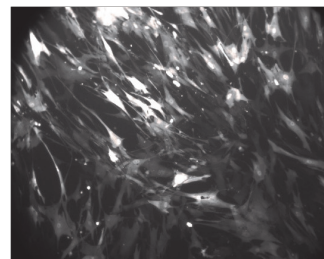
The DNA/RNA Delivery Core has significantly improved cloning protocols for its standard lentiviral expressing vectors for protein and shRNA expression, and Luciferase reporter vectors. We prepare linearized vectors in amounts sufficient for 4-6 ligation reactions. The improved Core cloning protocols are easy to use, even by first-time users. 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 work with you on delivery.

We have collaborated with Keratinocyte and Pathology Cores to develop protocols for the usage of lentiviruses in the models of NHEK organotypic raft cultures and colony assays. Examples of recent successes include reproducing the biological effects of glucocorticoid receptor delivered by lentivirus and of NOTCH delivered by LZRS retrovirus. RAFTs with overexpressed GR were significantly thinner than those infected with control vectors, while growth of NHEK colonies infected with GR was dramatically diminished.

Figure 1



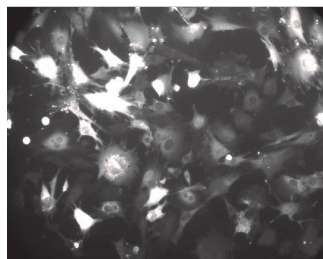
human primary renal mesangial cells



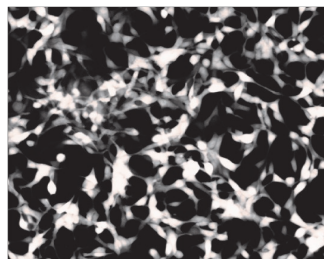
human primary smooth muscle cells



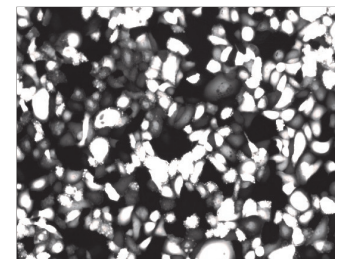
human transformed prostate carcinoma cell line PC3



mouse primary embryonic fibroblasts



human transformed prostate carcinoma cell line LNCaP



human transformed normal urothelium cells

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DNA/RNA Delivery Core – An Infectious Team

by Alexander Yemelyanov, PhD Continued from page 4

In a similar fashion, NHEK cells infected with NOTCH demonstrated significantly slower growth in the colony assay (Fig. 1).

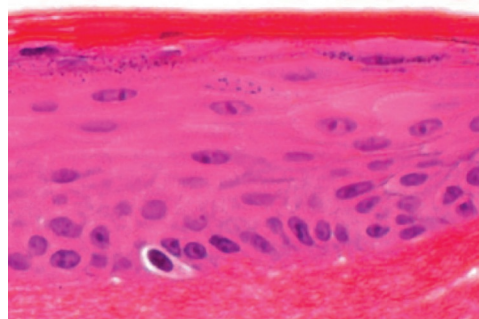
One of the major goals of the DNA/RNA Delivery Core this year and last year has been integration of products and services into activities of other NU core facilities. Indeed, we have established ongoing collaborations with the High Throughput / RNAi Core (Evanston Campus), which distributes shRNAs cloned into lentiviral expression vectors. These shRNA-vectors can be directly used to generate lentiviral stocks by our Core. The Core has also teamed with the Tumor Biology Core (Evanston

Campus) to generate stably infected cell lines for animal models. As the number of NU researchers who discover and utilize the DNA/RNA Delivery Core keeps increasing, we have branched out to apply our experience in infecting keratinocytes to a variety of non-keratinocyte primary cells and transformed cell lines of both mouse and human origin. We have achieved close to 100% infection rates on the following transformed cell lines: human prostate cancer cell lines (LNCaP, DU145, PC3), breast carcinoma cells lines (MDA-MB-231, MDA-MB-435, MDA-MB-468, and MCF-7), human urothelial cell lines (PDO7i, TEU1, TEU2), and colon carcinoma cells lines that are notoriously difficult to infect (Fig. 2). The Core's viral products have also successfully transduced mouse primary embryonic fibroblast, human primary smooth muscle cells, primary human renal mesangial cells and primary mouse keratinocytes (Fig. 2).

We are always ready for new challenging projects on difficult-to-infect cells and we look forward to working with additional scientists at NU who find delivery to be a potential dilemma. Feel free to contact us directly at a-yemelyanov@northwestern.edu and i-budunova@northwestern.edu. We may already have what you need for your research!

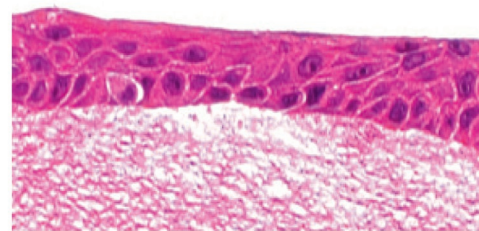
Figure 2

A.1



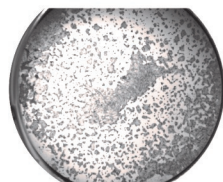
NHEK infected with control lentivirus

A.2

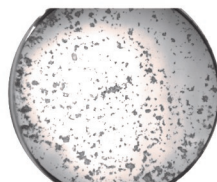


NHEK infected with GR expressing lentivirus

B.1

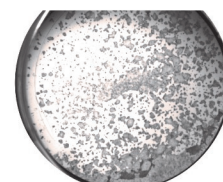


Control lentivirus

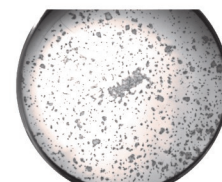


Lentivirus expressing GR

B.2



Control retrovirus



Retrovirus expressing active form of Notch

Would you like to receive updates on NU-SDRC news and upcoming events?

If so, join the new NU-SDRC listserv!

To join simply send an email to Betsy Cutcher at e-cutcher@northwestern.edu.



Getting to Know the SDRC featuring Hanz Blatt and Sunny Yang

by Betsy Cutcher, MSW

You'll notice a new face at the SDRC Pathology Core. As we bade a fond farewell to our Pathology Core Research Technician, Hanz Blatt, we welcomed a new addition to our team, Sunny Yang. Let's learn a bit more about them both....

Let's see where life is taking Hanz.....

- **What is your next step after the SDRC?**

I will be attending Loyola University to complete a Master's degree Medical Science.

- **What are you long term goals after you complete your Master's?**

To enter medical school and pursue a career as a physician-scientist, translating research to patient care.

- **Any parting words?**

My experience here has been both rewarding and challenging. The people I have come to know and care for in Dermatology have impressed upon me the values of scientific exploration. The knowledge and work ethic I have learned from my colleges will help me to achieve my future aspirations.



Let's get to know Sunny.....

- **Where is your hometown?**

I was born in Changsha, which is the capital city of Hunan in south-central China.

- **What brought you to Chicago?**

My family recently moved to here. I really like the Chicago city life, so I decided to make the move as well.

- **What do you like to do in your spare time?**

I'm an avid badminton fan and spend a lot of time honing my skills.

- **What degrees do you have and where did you obtain them?**

I received a Bachelor's of Science in Biotechnology from the Hunan Agricultural University in China and more recently a Master 's of Science and Medical Neuroscience from Indiana University.

- **Where did you work before and in what type of research?**

I previously worked at Indiana University in the Cardiovascular Disease Group. Our research focused on hypertension.

- **What attracted you to the Northwestern University Skin Disease Research Center?**

The interesting research and state-of-the-art facilities. I am looking forward to working for such a prestigious center and university.

- **What are your research interests?**

Histology, immunology and molecular biology.

Our new Pathology Core Technician, Sunny, can be reached via email shuangni.yang@northwestern.edu or phone 312-503-4192.



Congratulations to our new 2011-2012 Pilot and Feasibility study award recipients!

The NU-SDRC Pilot and Feasibility (P&F) study grants are a vehicle to encourage senior investigators without experience in epithelial biology to consider epithelial cell-related research initiatives that are natural tangents to their ongoing research. The Pilot and Feasibility Program is also a mechanism whereby junior investigators from several departments within the institution can initiate new projects that involve epithelial cell research. The NU-SDRC awards four P&Fs per year with the ultimate goal of generating sufficient preliminary data for the investigator to obtain extramural funding at the end of the funding period. Of the last group of recipients, two successfully obtained NIH support and a third received a sizeable foundation award to continue the studies. The NU-SDRC is proud to announce our 2011-2012 P&F recipients: Melissa Brown, PhD; Brian Mitchell, PhD; William J. Muller, MD, PhD; and Christian Stehlik, PhD.



P&F #5: **Melissa Brown, PhD**, Professor in the Department of Microbiology-Immunology in the Feinberg School of Medicine at Northwestern University, "**Mechanisms Underlying Adverse Cutaneous Effects Of The Anti-Cancer Drug, Bortezomib**". Dr. Brown is performing a series of studies involving the proteasome inhibitor Bortezomib (BZ), an anti-cancer drug used in the treatment of multiple myeloma. BZ has been found to have several adverse effects, particularly in the skin. Dr. Brown hypothesizes that these cutaneous side effects relate to mast cell recruitment to the skin. She therefore will test this hypothesis in Aim 1 and will also assess whether mast cell stabilizers might have utility in blocking the toxic effects of BZ. In Aim 2, Dr. Brown proposes to assay mast cell recruitment to the skin in patients being treated with BZ.

P&F #6: **Brian Mitchell, PhD**, Assistant Professor in the Department of Cell and Molecular Biology in the Feinberg School of Medicine at Northwestern University, "**The regulation of cell migration and intercalation through keratinocytes**". Dr. Mitchell is proposing to develop the experimentally pliable skin of *Xenopus* embryos as a model system for dissecting the molecular mechanisms via which cells break through and re-establish junctional barriers. *Xenopus* skin is an ideal system for this analysis since it consists of two distinct layers, the outer keratinocytes, and an inner basal layer composed of precursor cells with the ability to differentiate into either multi-ciliated cells or ionocytes. During development, these inner layer cells undergo a stereotyped migration event in which they intercalate into the keratinocyte layer. Dr. Mitchell proposes to define the complex cell biological processes which underlie how skin cells migrate in a directed manner, break down cell-cell junctions of the outer keratinocytes and stop their migration, forming new cell-cell junctions.

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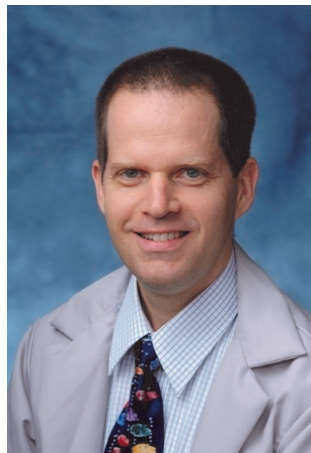


Lecture Series Highlight: *Fibrosis and Repair Group*

This group meets to share latest findings from various NU investigators, and an occasional guest, on cellular mechanisms by which wound heals or organ becomes fibrotic. The meeting provides a great opportunity to meet and discuss with NU research community who have similar scientific interests at various levels, from PI to post-doc and clinical fellows. **FRG meets the first Tuesday of the month from 9:00-10:15am in the Morton Medical Research Building, 310 E. Superior Street, Room #4-672.** For additional information on upcoming speakers contact Tomoko Hayashida at hayashida@northwestern.edu.

Congratulations to our new 2011-2012 Pilot and Feasibility study award recipients!

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P&F #7: **William J. Muller, MD, PhD**, Assistant Professor in the Department of Pediatrics in the Feinberg School of Medicine at Northwestern, "**Keratinocyte innate immune responses after herpes simplex virus infection**". Evidence from studies in mice and in tissue culture models suggests that the interaction of herpes simplex virus with one of its principal receptors, the herpes virus entry mediator (HVEM), influences early events in innate immune signaling. His overall hypothesis is that virus interaction with HVEM alters this signaling to promote viral replication and establishment of infection. Using virus which is altered to abolish interaction with HVEM, the Muller lab will use molecular techniques to measure the induction and translation of innate genes in human keratinocytes after infection and examine how this is influenced by HVEM signaling. They then plan to confirm and extend these results in assays using wild-type virus and cells lacking HVEM signaling, either murine cells with disruption in the HVEM gene, or human cells with reduced HVEM expression through gene knockdown.

P&F #8: **Christian Stehlik, PhD**, Assistant Professor in the Department of Medicine, Division of Rheumatology in the Feinberg School of Medicine at Northwestern University, "**Innate immune host defense function of keratinocytes**". Dr. Stehlik plans to characterize a novel innate immune function of keratinocytes by testing the hypothesis that select Nucleotide Oligomerization Domain receptors (NLRs) are able to promote inflammasome activation in keratinocytes upon sensing pathogen infection of the skin and to subsequently recruit phagocytes. He further hypothesizes that dysregulation of this system contributes to the excessive production of IL-1 β in inflammatory skin disease. Dr. Stehlik's first Aim is to assess the expression of inflammasome markers in skin cells and 3D raft cultures. The second Aim is of higher risk and will deal with mutations in inflammasome components that may trigger inflammasome activation and hence inflammatory disease.



Mark your calendars, upcoming events at the NU-SDRC!

In conjunction with the Department of Dermatology, the NU-SDRC hosts speakers from around the globe for special lectures throughout the year. Below you will find a few of the upcoming lectures which all are welcome to attend:

- **Tuesday, April 3, 2012: Ethan A. Lerner, MD, PhD**, Associate Professor of Dermatology at Harvard Medical School will be presenting a basic science lecture on Tuesday, April 3rd, 2012 in the afternoon regarding his work with PAR-2, and then the Dr. Donald Levin Lecture on Pruritus from 10:45- 11:45 am at the 676 North St. Clair building, Suite 1600 on Wednesday, April 4, 2012.
- **Tuesday, October 2, 2012:** The SDRC will be sponsoring **Dr. Paul A. Khavari, MD, PhD**, Professor and Chair of Dermatology at Stanford University for an October 2nd, 2012 LLS presentation. Stay tuned for additional details on Dr. Khavari's lecture. For a full list of upcoming LLS speakers contact Steven Anderson at sja314@northwestern.edu.