



April 2012

Office of Education, Division of Intramural Research
National Heart, Lung, and Blood Institute
FELLOWS NEWSLETTER

The Fellows Newsletter is published monthly by the Office of Education, Division of Intramural Research, National Heart, Lung, and Blood Institute and distributed to NHLBI DIR members to promote the interest of DIR Fellows.

Office of Education, DIR, NHLBI

Herbert M. Geller, Ph.D., Director
Angela N. Theofilos, Program Coord.
Aurora J. Taylor, Program Coord.

DIREducation@nhlbi.nih.gov

Building 10, Room 6N248
Tel: 301-451-9440

Fellows Advisory Committee

Kang Chen, LBC
Pradeep Dagur, HB
Alicia Evangelista, LCP
Jahda Hill, LMG
Zhiping Jiang, LPCD
Mohit Kashyap, LMI
Cory Lago, TMB
Jonathan Lam, TMB
Mark McDermott, LKEM
Kenneth Myers, LCTM
Attila Nagy, LMP
Cynthia St. Hilaire, MMC
Aibin Wang, LMC
Lu Wang, LMI
Thomas Winkler, HB
Julie Wu, TMB
Yingfan Zhang, LMC
Hang Zhao, LB

From the Director of the Office of Education

After all the pre-event jitters, we had what was, in my humble opinion, the best DIR Scientific Retreat ever. Zena Werb served double duty - she gave the Keynote talk on Wednesday evening and gave a great scientific talk on Thursday Morning. Alexandra Newton gave a great talk about protein kinase C on Friday. The quality of the fellows talks and posters was outstanding. Even the weather was ideal for facilitating informal communications. Even though we just finished this retreat, we'll soon start planning the next one. If you would like to be part of the process, please volunteer for the Fellows Advisory Committee. In addition, we are eager to have your suggestions for potential keynote and scientific speakers. And please send us any ideas you may have about how to improve the retreat!



Dr. Werb's Advice for the Young Scientist

By Herbert Geller, Ph.D.

Dr. Zena Werb's keynote talk was a short synopsis of her outstanding career, followed by a series of observations that she felt led to her success and then a most interesting question and answer period. She noted that when she evaluates someone's C.V. for a potential faculty position, she looks for several things:

Early publications: if your major project will take some time to develop, she advises writing either methods papers or reviews to get your name recognition and demonstrate your varied abilities.

Communication and networking: give talks – at journal clubs, or research in progress seminars, and reach out to postdocs in the next lab.

Mentoring: supervise a summer student or a postbac. These items on your c.v. show that you can supervise people.

Cont'd on page 3

<http://dir-intranet.nhlbi.nih.gov/oe/>

THE SCIENCE BEAT

By Daniel Kraushaar, Ph.D.

Yu, P. P., Santiago, L. Y., Katagiri, Y., & Geller, H. M. (2012). Myosin II activity regulates neurite outgrowth and guidance in response to chondroitin sulfate proteoglycans. *Journal of Neurochemistry* 120, 1117-1128.

Injury of the central nervous system (CNS) is followed by the formation of glial scars that expose neurons to a microenvironment that is inhibitory to neuronal growth and nerve regeneration. Much clinical and basic research is currently undertaken with the aim of restoring axonal self-repair and regeneration in the CNS. One major contributor to inhibition of axonal growth is the abundance of chondroitin sulfate proteoglycans (CSPGs) – negatively charged glycoproteins that are present in the extracellular matrix of the CNS and which act as potent repellents of neuronal guidance. The mechanisms by which CSPGs alter the response of neurons to neuronal guidance cues will ultimately rely on the reorganization of cytoskeletal components such as actin and tubulin but are currently not well understood. The NHLBI study by Yu *et al.* investigated the mechanisms that underlie changes in non-muscle myosin II (myosin II), an actin-binding protein, upon exposure to CSPGs. To this end, Yu and coworkers used cerebellar granule neurons and *in vitro* neurite guidance and neurite outgrowth assays that served to examine and track neuronal growth responses.

In their first set of experiments, neurons were plated onto either control poly-L-lysine (PLL) -coated coverslips or coverslips spotted with CSPGs mixed with Texas Red that allowed for the visualization of CSPG-rich regions. As the CSPG concentration in the spots increased, the number of neurites that crossed over into the spot decreased substantially and demonstrated the repellent action of CSPGs onto neurite crossing. In order to test whether myosin II activity may be affected by CSPGs and associated with reduced neurite guidance, the effect of CSPG exposure on the phosphorylation status of the myosin regulatory light chain, which regulates myosin II activity, was examined. Immunostaining revealed that myosin II phosphorylation was induced when neurons were plated onto substrate rich in CSPGs, especially in growth cones. Only low levels of phosphorylation were observed in neurons plated on PLL substrate. An increase in myosin II phosphorylation was also seen when neurons were exposed to soluble CSPGs indicating that

CSPG-mediated activation of myosin II may underlie the inhibition of neurite guidance. In the light of these results, experiments were carried out that were aimed at understanding whether inhibition of myosin II could overcome the CSPG-mediated inhibition of neurite crossing. Blebbistatin treatment, which selectively inhibits the ATPase activity of myosin II, significantly promoted the crossing of neurites into CSPG spots and suggested that myosin II inactivation can overcome CSPG-mediated inhibition of neurite guidance. Among the three isoforms of myosin II, myosin IIA and myosin IIB are the predominant isoforms in neurons. Knock-downs of myosin IIA and myosin IIB both increased neurite crossing and showed that both isoforms of myosin II are involved in this process.

Next, Yu *et al.* tested whether inhibition of myosin II activity by CSPGs affects solely neurite guidance *per se* or other neuronal growth processes such as neurite initiation and elongation. A quantitative analysis of the percentage of neurons with neurites extending longer than one cell body diameter showed that a smaller percentage of neurons initiated neurite outgrowths when cultured on CSPGs compared to PLL control substrates. Pre-treatment with blebbistatin dramatically increased neurite initiation both on PLL and CSPG substrates. Time-lapse recordings of neurite growth revealed that blebbistatin treatment accelerated neurite initiation with neurite extension becoming visible already within 90 minutes after plating on both PLL and CSPG substrates. DMSO treated neurons in contrast had spread but not yet formed neurites within the same time period. Furthermore, measurements of neurite length 24 hours after plating showed that on average neurites were shorter on CSPGs. Treatment with blebbistatin resulted in substantially longer neurites. Together these results revealed that myosin II plays a pivotal role in initiation, outgrowth and guidance of neurites in response to inhibitory CSPGs. This work will surely prompt further studies into elucidating the signaling cascade that is triggered by CSPGs and examination as to whether protein or carbohydrate are required for induction of myosin II activity. The use of specific pharmacological inhibitors of myosin II may provide means to promote axonal regeneration upon CNS injury and to overcome CSPG-mediated inhibition of neuronal growth.

Photos from the 10th Annual NHLBI DIR Scientific Retreat



Congratulations to the Poster Winners

Alex Jares, Post Bac



Brad Webster, Graduate Student



Brian Galetta, Post Doc



Raul Covian Garcia, Staff Scientist



New NHLBI Fellow



Ambika Bumb, Ph.D., is a new Post Doctoral fellow in the Laboratory of Molecular Biophysics under Dr. Keir Neuman. Dr. Bumb received her Ph.D in Medical Engineering from the University of Oxford. She held her first Post Doctoral position with the National Cancer Institute in the Radiology Oncology Branch. Dr. Bumb is currently researching the development of novel near-infrared nanodiamond probes .

Getting grants and fellowships: this is not just to obtain money, but to show that you can compete and get funded and are aware of the system. Even if you fail, you may learn from the process.

Teaching: if you want a position that requires teaching try to volunteer or teach a class at FAES. Sit down and write a teaching vision/portfolio.

Make a more scientific c.v.: try for the "big" publication, but make sure that you have productivity. If only have one publication, then it raises question.

Develop a project to take with you: start a project that is not of direct interest to the lab, and that can be yours.

Target your audience: if you want a career in Biotech, then reach out to Biotech scientists. Invite them to speak here at NHLBI, go to their posters at meetings where you meet the people doing the science, since they can get your c.v. placed (and, at many companies, they get money if you get hired).

Overall, she suggested that you need to do what makes you happy if you are going to be successful. Thus, if your life style is important, you may not want to have a low-paying academic job, and might want to consider a career where you are not the P.I. Overall, you may need to compromise, but you need to have a sense of what your bottom line will be.

The bottom line is you need to be your own best mentor and do the best for yourself!

Recent Publications by NHLBI Fellows

- Bish, L. T., Sleeper, M. M., Forbes, S. C., Wang, B. J., Reynolds, C., Singletary, G. E., Trafny, D., Morine, K. J., Sanmiguel, J., Cecchini, S., **Virag, T.**, Vulin, A., Beley, C., Bogan, J., Wilson, J. M., Vandenborne, K., Kornegay, J. N., Walter, G. A., Kotin, R. M., Garcia, L., & Sweeney, H. L. (2012). Long-term Restoration of Cardiac Dystrophin Expression in Golden Retriever Muscular Dystrophy Following rAAV6-mediated Exon Skipping. *Mol. Ther.* *20*, 580-589.
- Chepelev, I., **Wei, G.**, Wangsa, D., Tang, Q. S., & Zhao, K. J. (2012). Characterization of genome-wide enhancer-promoter interactions reveals co-expression of interacting genes and modes of higher order chromatin organization. *Cell Res.* *22*, 490-503.
- Degheidy, H. A., Venzon, D. J., **Farooqui, M. Z. H.**, Abbasi, F., Arthur, D. C., Wilson, W. H., Wiestner, A., Stetler-Stevenson, M. A., & Marti, G. E. (2012). Combined normal donor and CLL: Single tube ZAP-70 analysis. *Cytometry B. Clin. Cytom.* *82B*, 67-77.
- Larochelle, A., Gillette, J. M., Desmond, R., **Ichwan, B.**, Cantilena, A., Cerf, A., Barrett, A. J., Wayne, A. S., Lippincott-Schwartz, J., & Dunbar, C. E. (2012). Bone marrow homing and engraftment of human hematopoietic stem and progenitor cells is mediated by a polarized membrane domain. *Blood* *119*, 1848-1855..
- Mciver, Z. A.**, Melenhorst, J. J., **Grim, A.**, Naguib, N., Weber, G., Fellowes, V., Khuu, H., Stroncek, D. S., Leitman, S. F., Battiwalla, M., & Barrett, A. J. (2011). Immune Reconstitution in Recipients of Photodepleted HLA-Identical Sibling Donor Stem Cell Transplantations: T Cell Subset Frequencies Predict Outcome. *Biol. Blood Marrow Transpl.* *17*, 1846-1854.
- Mielke, S., **Mciver, Z. A.**, **Shenoy, A.**, Fellowes, V., Khuu, H., Stroncek, D. F., Leitman, S. F., Childs, R., Battiwalla, M., Koklanaris, E., Haggerty, J., Savani, B. N., Rezvani, K., & Barrett, A. J. (2011). Selectively T Cell-Depleted Allografts from HLA-Matched Sibling Donors Followed by Low-Dose Posttransplantation Immunosuppression to Improve Transplantation Outcome in Patients with Hematologic Malignancies. *Biol. Blood Marrow Transpl.* *17*, 1855-1861.
- Sable, C. A., **Aliyu, Z. Y.**, Dham, N., Nouraie, M., Sachdev, V., Sidenko, S., Miasnikova, G. Y., Polyakova, L. A., Sergueeva, A. I., Okhotin, D. J., Bushuev, V., Remaley, A. T., Niu, X. M., Castro, O. L., Gladwin, M. T., Kato, G. J., Prchal, J. T., & Gordeuk, V. R. (2012). Pulmonary artery pressure and iron deficiency in patients with upregulation of hypoxia sensing due to homozygous VHLR200W mutation (Chuvash polycythemia). *Haematologica* *97*, 193-200.
- Pfefferkorn, C. M.**, F. Heinrich, A. J. Sodt, A. S. Maltsev, R. W. Pastor, and J. C. Lee. (2012). Depth of alpha-synuclein in a bilayer determined by fluorescence, neutron reflectometry, and computation. *Biophys. J.* *102*, 613-621.
- Seabold, G. K., Wang, P. Y., Petralia, R. S., Chang, K., Zhou, A., **McDermott, M. I.**, Wang, Y. X., Milgram, S. L., & Wenthold, R. J. (2012). Dileucine and PDZ-binding Motifs Mediate Synaptic Adhesion-like Molecule 1 (SALM1) Trafficking in Hippocampal Neurons. *J. Biol. Chem.* *287*, 4470-4484.
- Vazquez, E., **Sethi, A. A.**, Freeman, L., Zalos, G., **Chaudhry, H.**, Haser, E., **Aicher, B. O.**, Aponte, A., Gucek, M., Kato, G. J., Waclawiw, M. A., Remaley, A. T., & Cannon, R. O. (2012). High-Density Lipoprotein Cholesterol Efflux, Nitration of Apolipoprotein A-I, and Endothelial Function in Obese Women. *Am. J. Cardiol.* *109*, 527-532.
- Yu, P. P.**, **Santiago, L. Y.**, Katagiri, Y., & Geller, H. M. (2012). Myosin II activity regulates neurite outgrowth and guidance in response to chondroitin sulfate proteoglycans. *Journal of Neurochemistry* *120*, 1117-1128.
- Zhang, J. H., Chen, Z. L., Fritz, J. H., **Rochman, Y.**, Leonard, W. J., Gommerman, J. L., Plumb, A. W., Abraham, N., & Croy, B. A. (2012). Unusual timing of CD127 expression by mouse uterine natural killer cells. *J. Leukoc. Biol.* *91*, 417-426.
- Zhou, Z. L., **Yu, P. P.**, Geller, H. M., & Ober, C. K. (2012). The role of hydrogels with tethered acetylcholine functionality on the adhesion and viability of hippocampal neurons and glial cells. *Biomaterials* *33*, 2473-2481.
- Zhu, X.**, **Ahmad, S. M.**, Aboukhalil, A., Busser, B. W., Kim, Y., Tansey, T. R., Haimovich, A., Jeffries, N., Bulyk, M. L. and Michelson, A. M. (2012). Differential regulation of mesodermal gene expression by *Drosophila* cell type-specific Forkhead transcription factors. *Development* *139*, 1457-1466.