Annual Inter-PEN Meeting

Boston, MA

October 19-20, 2012

Our Mission

The goal of NHLBI Programs of Excellence in Nanotechnology is to develop nanotechnology – based tools for the diagnosis and treatment of heart, lung, and blood diseases, and to move the translation of these technologies towards clinical application. The program brings together multi-disciplinary teams from the biological, physical, and clinical sciences for the focused development and testing of nanoscale devices or devices with nanoscale components, and applies them to cardiovascular, hematopoietic, and pulmonary diseases. The program also develops investigators with the interdisciplinary skills to apply nanotechnology to heart, lung, and blood disease problems.

Contents…

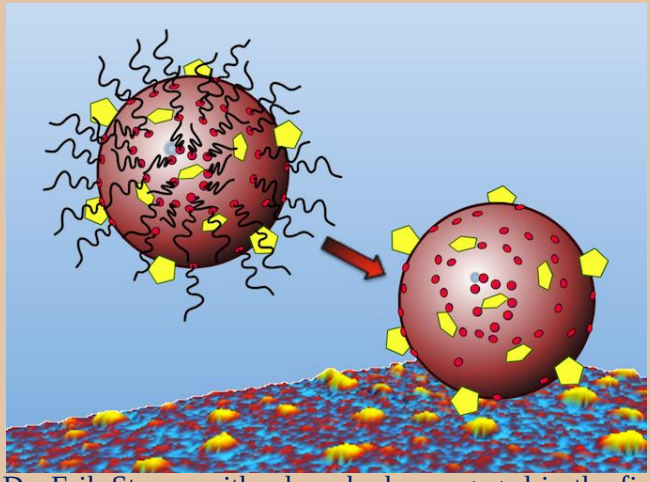
PEN Spotlight On…

Willem Mulder, Ph.D. – Mount Sinai School of Medicine

Dr. Willem Mulder is an Associate Professor of Radiology at Mount Sinai School of Medicine (MSSM) in New York. Dr. Mulder, a chemist by training, and received his Ph.D. in Biomedical Engineering from the Eindhoven University of Technology in The Netherlands in 2006. Thereafter he moved to MSSM to found the Nanomedicine Laboratory within the Translational and Molecular Imaging Institute (TMII), directed by Dr. Zahi Fayad.

Currently, Dr. Mulder directs TMII's Nanomedicine Laboratory (Nano-TMII) and his research is focused on the development, preclinical testing and translation of nanoparticle imaging agents and nanoparticle therapies for cancer and cardiovascular disease. Because the Nanomedicine Laboratory resides within an imaging institute, the research has a strong emphasis on in vivo imaging, which allows the visualization of nanoparticle biodistribution and targeting as well as nanotherapeutic efficacy.

His team consists of 5 post doctoral fellows, 4 Ph.D. students, 2 technicians and on average 2-4 (visiting) undergraduate students. The nanomedicine research projectsat Nano-TMII are diverse, highly multidisciplinary and imaging oriented. They range from the basic development of nanoparticle platforms with 'smart' coatings (figure) or as 'smart' reporters, to full-blown preclinical mouse nanotherapy studies, all the way to translational studies with rabbit models.



To facilitate the translation of nanotherapy for atherosclerosis patients, Dr. Mulder also holds a strategic appointment at the Academic Medical Center (AMC) in Amsterdam. At the AMC he collaborates with a team of clinical trialists, led by Dr. Erik Stroes, with whom he has engaged in the first clinical trial in cardiovascular disease patients using an anti-inflammatory nanotherapeutic approach directly targeting the atherosclerotic plaque.

The research at Nano-TMII is supported by several NIH grants, including an R01 through the National Cancer Institute and the Program of Excellence in Nanotechnology (directed by Drs. Zahi Fayad and Robert Langer) through the National Heart Lung and Blood Institute. For the latter program Dr. Mulder serves as a co-Principal Investigator on a project that revolves around theranostic cardiovascular nanomedicine. The Dutch Science Foundation (NOW) supports research that is aimed at developing the latest generation quantum dot probes.

Dr. Mulder has authored more than 70 papers and 7 book chapters, has trained over 20 young scientists, and is a regular invited speaker at international conferences. His work has been highlighted in an article entitled "From the Lab, a New Weapon Against Cholesterol" in The New York Times in 2009 and, more recently, in The Daily News at the 2011 AHA meeting in Orlando.

The "Spotlight" rotates between the PENs. If someone on your PEN has made a significant contribution to your PEN's success and you would like to recognize their work, please send a brief biography to Terry Sharp at sharpt@mir.wustl.edu.

MSSM, MIT, BWH, Columbia, NYU

Translational Nanomedical Therapies for Cardiac and Vascular Diseases

Principal Investigator – Zahi A. Fayad, Ph.D.
Co-Principal Investigator – Robert S. Langer, Ph.D.

Increased local expression of SCF recruits progenitor cells to damaged hearts and boosts repair

Elisa Yaniz-Galende & Roger J. Hajjar

When the heart tissue is damaged by a myocardial infarction, cardiac progenitor cells are recruited to the injury site to aid repair. But it is all too clear from the number of heart attack survivors suffering from hypertrophy and heart failure, that this repair process is limited. We wondered whether this limitation could be overcome by increasing the number of progenitor cells to the site of injury. We sought to develop a clinically relevant local expression system for the membrane-bound stem cell factor (SCF) isoform to optimally stimulate cardiac repair after experimental MI. SCF is the ligand for the cell-surface receptor c-kit, expressed on progenitor cells, and it both recruits progenitor cells and promotes their proliferation.

We induced myocardial infarctions in rats and then injected the peri-infarct area with adenovirus vectors carrying the gene for stem cell factor (SCF). One week after the infarctions, SCF-overexpressing hearts had recruited higher numbers of progenitor cells. And a couple of months after that, the SCF hearts exhibited, decreased fibrosis, less left ventricular hypertrophy, and, importantly, improved function as shown in **Figure 1**. The rats themselves also survived longer as shown in **Figure 2**.

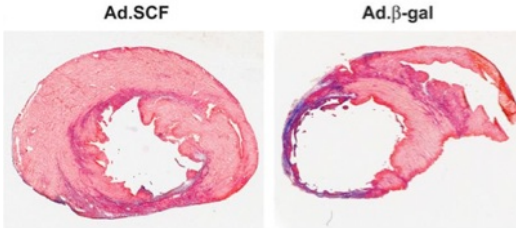


Figure 1: Representative Masson trichrome–stained myocardial sections from control and SCF-treated rats 1 month post-myocardial infarction (MI). Blue, scar tissue; red, viable myocardium.

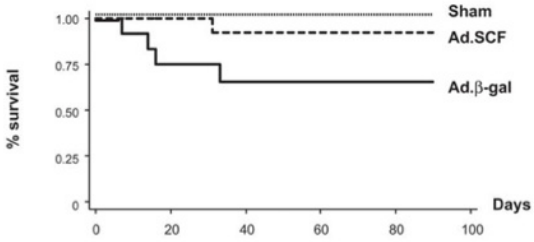


Figure 2: Kaplan–Meier survival analysis in sham (n=5), adenoviruses containing β-gal and green fluorescent protein (Ad.β-gal)–treated group (n=10), and adenoviruses expressing SCF membrane-bound form and green fluorescent protein (Ad.SCF)-treated groups (n=13).

In the present study, we used intramyocardial SCF adenoviral gene transfer to enhance cardiac repair after LAD ligation by recruiting and expanding resident c-kit+ cardiac progenitor/stem cells. Our studies have shown several significant findings. Using transient SCF expression, we observed an increase in cardiac c-kit+ cell recruitment after MI. Increased c-kit+ CPC population in SCF hearts is demonstrated by 2 independent techniques: cell counting by histology and sorting flow cytometry. These CPCs are a mixed population containing not only CSCs and precursors but also cells committed to other lineages. Although the majority population of c-kit+ cells are presumably endogenous, we do observe a high enrichment of c-kit+CD45+ fraction post-MI, suggesting a BM origin for many of the CPCs isolated. This conclusion is also consistent with the studies reporting that BM progenitor cells are mobilized after MI, homing to injured areas promoting cardiac repair. Regardless of cell origin, the increase in c-kit+ cells in SCF-treated hearts, whether CSC, BM cells, or a combination, does mediate a beneficial effect on survival, cardiac structure, and ventricular function after MI. This study suggests that a similar type of gene therapy approach might be possible as a cardiac regenerative strategy in patients.

Hot Topic

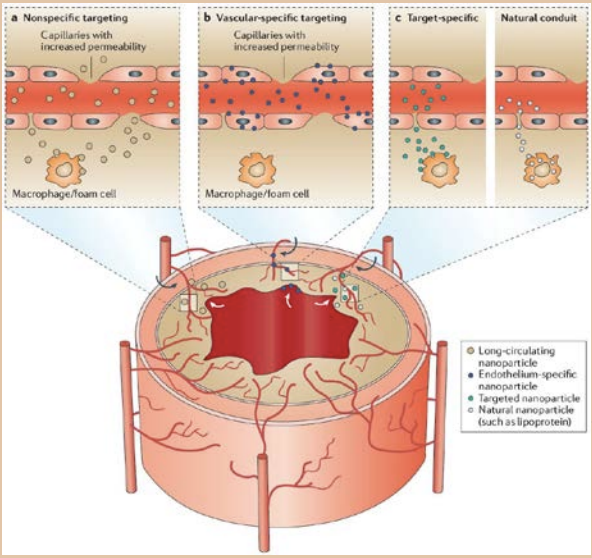


Figure: Lobatto et al. Nat Rev Drug Discov. 2011.

Our lab is interested in targeting atherosclerotic plaques with long-circulating controlled release nanoparticles to enable site-specific anti-inflammatory drug delivery. Based on a vast amount of experience in cancer, the current belief is that long-circulating nanoparticles extravasate from the circulation and into the atherosclerotic plaque through dysfunctional endothelium at the luminal side of the plaque, as well as through immature neovessels with a leaky endothelium that extend from the vasa vasorum into the base of the plaque (**Figure**). Although it has been shown that nanoparticles accumulate in plaques, the mechanism of uptake has not been thoroughly studied. To further elucidate atherosclerotic plaque targeting we are conducting a study in atherosclerotic rabbits where we visualize nanoparticle targeting kinetics by multimodality imaging experiments. Imaging techniques include 3 dimensional dynamic contrast enhanced magnetic resonance imaging (MRI), micro computed tomography (μCT), near infrared fluorescence (NIRF) imaging, as well as microscopic techniques.

Awards

Dr. Willem Mulder, one of the co-directors of Project 3 of the Mount Sinai-MIT PEN was promoted to Associate Professor of Radiology in September 2012.

Mark Lobatto, a student from the Mount Sinai-MIT group was named the runner up of the Vascular Biology Year prize by the Vascular Biology Working Group of the Netherlands, which enabled him to present his work on atherosclerosis nanotherapy at the AHA meeting in Los Angeles in November 2012.

Save the Date…

2013 Inter-PEN Meeting
October 18-19, 2013
Hosted by:
Mount Sinai School of Medicine

MGH, BWH, BI, Harvard, MIT

Translational Program of Excellence in Nanotechnology

Principal Investigator – Ralph Weissleder, Ph.D.

Photocleavable DNA Barcode–Antibody Conjugates Allow Sensitive and Multiplexed Protein Analysis in Single Cells[1]

By Jason R. McCarthy and Ralph Weissleder

The ability to profile proteins and antigens in single cells allows for the investigation of the role of cellular heterogeneity in disease progression, stem cell differentiation, response to drugs, and other cellular signaling processes. This is becoming increasingly important in a number of fields, including biological research, forensic science, and clinical diagnostics. In particular, molecular profiling and proteomic analysis of rare cells holds considerable promise for early disease detection and monitoring of treatment response. To this end, Dr. Weissleder and his team have investigated novel applications of DNA barcoding. DNA barcoding possesses several advantages, including commonly used amplification techniques (PCR), and the ability to generate infinite numbers of different barcodes. Although DNA barcoding has been used to detect soluble proteins, there have been few examples of its successful application to live cells.

To enable this technology, the researchers utilized a photocleavable linker moiety to enable barcode release, amplification and readout following target binding (**Figure 1**). This strategy initially used DNA barcode labeled antibodies to identify specific protein biomarkers. Once bound, the cells would be irradiated with 365 nm light, releasing the barcode, which is readily isolated and amplified via PCR. Gel electrophoresis can then be used to simultaneously detect and quantify multiple protein analytes from single cells.

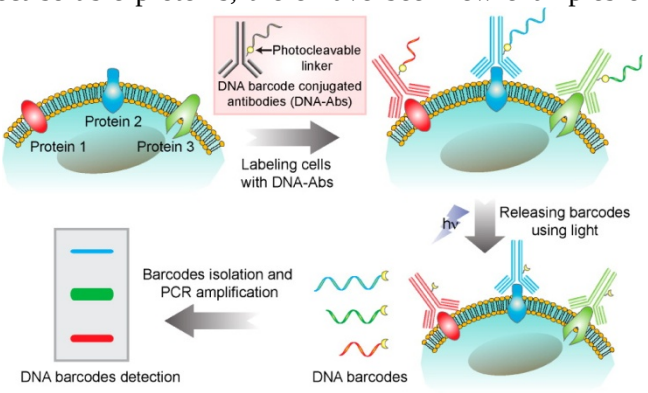


Figure 1. Schematic Illustration of the Light-Mediated Cellular Barcoding Strategy

Initial experiments with anti-HER2/neu antibodies modified with fluorescently labeled DNA demonstrated that the conjugates did indeed bind to HER2/neu overexpressing cells (SK-BR3), with the fluorescent decreasing significantly after irradiation. This technique was subsequently applied to SK-BR3 (HER2/neuhigh) and control fibroblast 3T3 (HER2/neulow) using an 85 base DNA barcode conjugated to the anti-HER2/neu antibody. After photocleavage, the DNA was amplified via 25 cycles of PCR. As expected, the target marker was only detectable after amplification on the cells presenting the antigen (SK-BR3), whereas the 3T3 cells did not generate detectable signal. These results were further validated by flow cytometry, which utilized far more cells per analysis (~105 cells for flow cytometry vs ~1 cell for this methodology).

The authors next investigated the detection threshold of this technology. Serial dilutions of SK-BR3 cells (104-100 cells per well) were targeted with the 85 base DNA barcode modified anti-HER2/neu antibody, photoreacted, amplified, and separated. Clear bands for the 85 base DNA were detected in all samples with intensities varying based upon the number of cells. This was further investigated with single cells, where wells containing one cell readily generated detectable bands on gels corresponding to the DNA barcodes.

In one last experiment, the researchers demonstrated the multiplexing of the DNA barcodes (80 base, 75 base, 55 base) for the simultaneous detection of EGFR, EpCAM, and HER2/neu in four different cell lines. As each of the barcodes contained identical sequences toward the 5′ and 3′ ends, the barcodes could then be simultaneously amplified by PCR

using a single set of primer pairs. As is shown in Figure 2, the biomarkers were readily distinguished from one another based upon barcode size, with levels clearly visible on the gel. The developed technique enables rapid, quantitative, multiplexed detection of protein expression in single live cells. Its robustness can be readily adapted to a number of other targets, including soluble proteins and pathogens. Ultimately, this is a relatively simple methodology that does not require complex purification steps, during which analytes are often lost.

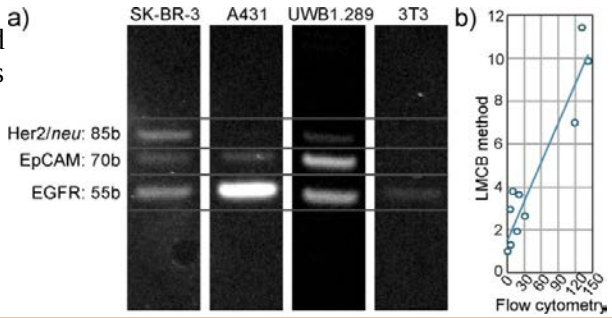


Figure 2. (a) Multiplexed protein detection using the method. Individual biomarker signals (corresponding to their expression levels) can be clearly distinguished from one other on the basis of barcode size. (b) Comparison of the results from (a) and data acquired using flow cytometry. For each biomarker, band intensities (normalized to control 3T3 cells) from the gel were plotted against fluorescence intensities (normalized to control 3T3 cells) from flow cytometry (R2 = 0.90).

References

(1) Agasti; S.S., Liong; M., Peterson; V.M., Lee; H., Weissleder; R. "Photocleavable DNA Barcode-Antibody Conjugates Allow Sensitive and Multiplexed Protein Analysis in Single Cells." J Am Chem Soc **2012**, In Press.

Hot Topic

The Hilderbrand lab (Center for Systems Biology, MGH) is currently exploring the potential for new photonic nanomaterials for diagnostic and imaging applications, with a focus on upconverting ceramic nanoparticles. Upconverting nanocrystals are excited in the NIR at 980 nm using low-power CW diode lasers, and depending on the composition, can have visible, far-red, or NIR emission at 550, 660, or 800 nm, respectively. The anti-Stokes excitation of these particles enables the near complete elimination of background autofluorescence signal in biological imaging experiments, and may also offer strategic advantages for a variety of diagnostic detection schemes.

Thank You

Thank you to all who participated in the 2012 Inter-PEN meeting. It was, as always, a wonderful collaborative environment.

A very special thanks to the Faculty and Staff at Massachusetts General Hospital for their hospitality. This event would not have been possible without **Ralph Weissleder**, **Jason McCarthy**, **Dianne Moschella**, and **Matthew Mues**.

Georgia Tech, Emory, UC-Davis

Center for Translational Cardiovascular Nanomedicine

Principal Investigator – Gang Bao, Ph.D.

Hot Topic

The Goodman lab is developing new unsaturated reagents for facile copper free reaction with organic azides for radiolabeling biologically active small molecules, peptides and nanoparticles with positron emitting radio-elements. The reagents are alternatives to existing alkyne reagents which require copper salts to facilitate the Huisgen 1,3-diploar cylcloaddition reaction (Click Reaction) for formation of 5-membered heterocycles.

Awards

Dr. Mark Goodman will receive a 2012 Distinguished Investigator Award by the Academy of Radiology Research on November 26, 2012 at the Radiological Society of North America Annual Meeting in Chicago Illinois. This honor recognizes individuals for their accomplishments in the field of imaging research that have had a profound impact on healthcare. Researchers who have been named a Distinguished Investigator have made significant contributions to the field.

Dr. Kathy Ferrara received the IEEE (Institute of Electical and Electronic Engineers) UFFC (Ultrasonics Ferroelectrics and Frequency Control) Achievement Award in October 2012 for long-term contributions to biomedical ultrasonics, molecular imaging, and image guided drug delivery.

ACPEN Announcement

by Robert Gropler, M.D.

Kari Alca has accepted another position at the Washington University School of Medicine. Even though this is a real loss to the ACPEN, the position is quite exciting and represents a wonderful opportunity for Kari. I know I speak for all of us that we certainly appreciate Kari's superb work on the ACPEN and will miss her.

Terry Sharp will assume all of Kari's duties on the ACPEN. Currently, Terry handles all of the contractual and budget aspects of the WU-PEN and is well versed in the PEN program's scientific goals, organizational demands and commitments. We are fortunate to have someone of Terry's talents and experience to step in. Please feel free to contact Terry to congratulate her on her new position.

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WUSTL, TAMU, UCSB, UTSW

Integrated Nanosystems for Diagnosis and Therapy

Co-Principal Investigators

Steven L. Brody, M.D., Robert J. Gropler, M.D., and Karen L Wooley, Ph.D.

Positron Emission Tomography Imaging of Chemokine Receptors in Vascular Injury Accelerated Atherosclerosis

Yongjian Liu, Richard Pierce, Hannah P. Luehmann, Terry L. Sharp, Michael J. Welch

Background: Atherosclerosis is the pathophysiologic process behind lethal cardiovascular diseases. It is inherently considered as a chronic inflammatory progression. Chemokines can strongly affect the initiation and progression of the atherosclerosis by controlling the trafficking of inflammatory cells in vivo through the interaction with their receptors. Some chemokine receptors were reported to play an important role in plaque development and stability. However, the diagnostic potential of chemokine receptors has not yet been explored. The purpose of this study was to develop a positron emitter radiolabeled probe to image the up-regulation of chemokine receptor in a wire-injury accelerated ApoE-/- mouse atherosclerosis model.

Methods: A virus macrophage inflammatory protein-II (vMIP-II) was used to image the up-regulation of multiple chemokine receptors through the conjugation with 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) for ^{64}Cu radiolabeling and positron emission tomography (PET). Imaging studies were performed at two and four weeks post injury in both wire-injured ApoE-/- and wild-type C57BL/6 mice. Competitive PET blocking studies with non-radiolabeled vMIP-II were performed to confirm the imaging specificity. Specific PET blocking with individual chemokine receptor antagonist was also carried out to verify the up-regulation of particular chemokine receptor. In contrast, [^{18}F]FDG PET imaging was performed in both models to evaluate tracer uptake. Immunohistochemistry on the injury and sham tissues were carried out to assess the up-regulation of chemokine receptors.

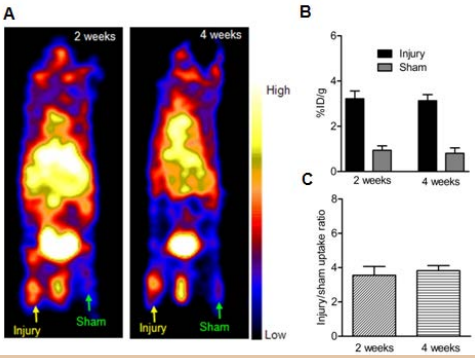

FIGURE 1. PET imaging (0-60 dynamic scan) of 64Cu-DOTA-vMIP-II in wire-injured ApoE-/- mice (**A**) showing accumulation of activity at the injury lesion, with little observed in contralateral sham-operated thigh at two weeks and four weeks post injury. ^{64}Cu-DOTA-vMIP-II accumulation at the injury and sham sites (**B**) and ^{64}Cu-DOTA-vMIP-II injury/sham uptake ratios (**C**) at the studied time points. *=p<0.001.

Results: [^{15}O]CO PET showed decreased blood volume in the femoral artery after the injury. ^{64}Cu-DOTA-vMIP-II exhibited fast in vivo pharmacokinetics with major renal clearance (**Figure 1A**). PET images showed specific accumulation around the injury site with consistent expression during the study period (**Figure 1B,C**). Quantitative analysis of tracer uptake at injury lesion in the ApoE-/- model showed three-fold increase compared to the sham-operated site (**Figure 1B**) and those obtained from injured wide-type mouse. [^{18}F]FDG PET showed significantly less tracer accumulation than ^{64}Cu-DOTA-vMIP-II with no difference observed between injury and sham sites. PET blocking studies with the co-injection of non-radiolabeled DOTA-vMIP-II showed significantly decreased tracer accumulation at injury lesion, indicating chemokine receptor-mediated ^{64}Cu-DOTA-vMIP-II uptake. Specific PET blocking of individual chemokine receptor verified the presence of 8 chemokine receptors (**Figure 2**), which were also confirmed by immunohistochemistry staining (**Figure 3**).

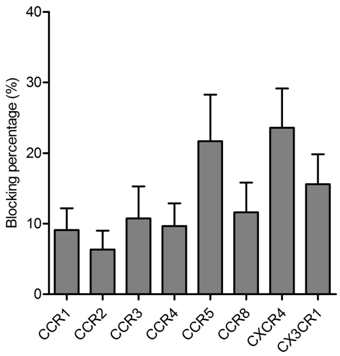

FIGURE 2. Individual chemokine receptors blocking percentage. Individual chemokine receptor antagonists were co-injected with 64Cu-DOTA-vMIP-II in ApoE-/- mice for PET imaging at three weeks post injury. The decrease of 64Cu-DOTA-vMIP-II uptake, compared to uptake observed at two weeks post injury with 64Cu-DOTA-vMIP-II, was calculated as blocking percentage. The blocking percentages for the eight chemokine receptor antagonists were in the range of 6.34±2.66% to 23.6±5.57%.

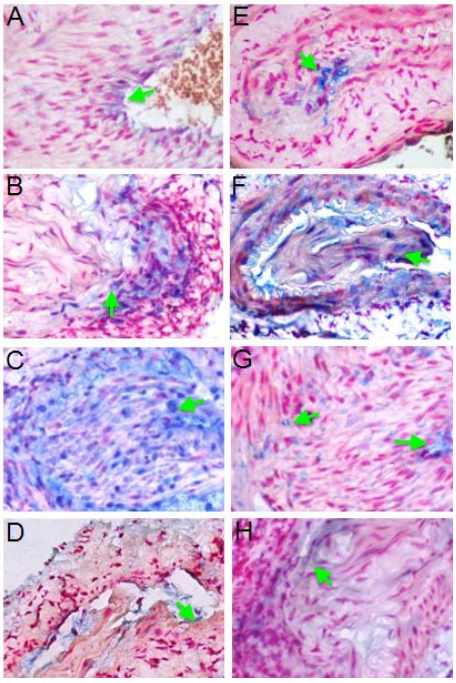

FIGURE 3. Immunohistochemistry of chemokine receptors: (**A**) CCR1 signal localized to the luminal surface in injured vessels, (**B**) CCR2-positive cells present in the medial layer of injured vessels, (**C**) CCR3 expression found throughout the walls of injured femoral arteries, (**D**) CCR4 localized to isolated cells at the luminal surface of injured vessels, (**E**) CCR5 signal present in cells medial to the internal elastic lamina of injured vessels, (**F**) CCR8-positive cells found throughout the walls of injured vessels, (**G**) CX3CR1-positive cells scattered through the walls of injured arteries, and (**H**) CXCR4 signal found in scattered vessel wall cells with injury.

Conclusion: ^{64}Cu-DOTA-vMIP-II was proven a sensitive and useful PET imaging probe for the detection of eight up-regulated chemokine receptors in an injury accelerated atherosclerosis model.

Hot Topic

Development of Degradable Cationic, Poly(DL-lactic acid) based Polymer Nanoparticle Carriers for DNA Delivery to the Lung

The airway provides a direct route for administration of nanoparticles bearing therapeutic or diagnostic payloads to the lung, however optimization of nanoplatforms for intracellular delivery remains challenging. Toward this end, the Wooley group has generated a library of poly(DL-lactic acid) based degradable cSCK (Deg-cSCK) a cleavable crosslinker, capable of binding plasmid cDNA. Studies by the Brody lab have demonstrated that in vitro transfection efficiency of the degradable is superior to prior non-degradable versions of the cSCK and comparable to commercial lipid reagents. We have used intratracheal delivery in mouse lung to test transfection efficiency of this library of Deg-cSCKs and to study biodistribution. In vivo, gene reporter studies show high levels of transfection, with relatively lower inflammatory response, particularly when PEGylated forms of the Deg-cSCK are used. Current efforts are directed toward optimizing dosing schemes and evaluation of the delivery of therapeutic genes that can improve outcome from lung injury.

Aida Ibricevic, Sean P. Guntsen, Sandani Samarajeewa, Ritu Shrestha, Tianyi Cai, Yuefei Shen, Kvar Black, Yongjian Liu, John-Stephen Taylor, Karen L. Wooley and Steven L. Brody1,

Awards

Sandani Samarajeewa received the ACS Polymeric Materials: Science and Engineering TA Instruments Graduate Student Presentation Award, National ACS Meeting, Philadelphia, PA, August 2012 for her presentation titled "Development of Degradable Cationic Shell Crosslinked Nanoparticles toward Delivery of Nucleic Acids".

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