ANNUAL POSTER SESSION

ABSTRACT BOOK

January 18, 2017
Building 13 Lobby

MIT Center for Environmental Health Sciences
Supported by NIH-NIEHS Core Center Grant P30-ES002109

http://cehs.mit.edu/

Image courtesy of the Engelward Lab
Directors’ Welcome

Welcome to the 2017 Annual Center for Environmental Health Sciences (CEHS) Poster Session. This tradition is an annual gathering of the environmental health research communities of MIT and some of our sister institutions. There will be about 60 posters presented from the science and engineering laboratories affiliated with the CEHS.

As you know, the one important theme of the MIT CEHS is the study of the biological effects of exposure to environmental agents in order to understand, and predict, how such exposures affect human health. Moreover, by uncovering the mechanistic bases for environmental disease, sometimes we are able to leverage that understanding to delay or even prevent the development of disease in human populations. A second CEHS theme is the measurement of hazards in the environment and the study of how those hazards move in space and time. Often chemical hazards are monitored by novel homegrown technology. The Poster Session brings engineers, chemists and biologists together under one umbrella to share ideas and showcase new technology.

The Center collectively represents forty-three members from a total of nine MIT departments (in both the School of Science and the School of Engineering), plus one Harvard faculty member from the Harvard School of Public Health.

The success of the Center depends on the interactions of its membership and participation in such events as this one. We are delighted to see that so many of you have taken this opportunity to share your research with others whose interests address the environment and environmental causes of disease.

This year, through the generosity of the Myriam Marcelle Znaty Research Fund, we are able to offer cash prizes for outstanding posters. Prizes to be awarded are 1st place: $1,000; 2nd place: $500; and 3rd place: $200 plus CEHS mementos. Prizes will be awarded in each category to both graduate students and postdoctoral scholars.

We hope that in the coming year the Center will continue to grow and to serve the needs of its membership and together achieve our common goals.

John M. Essigmann
Director

Bevin P. Engelward
Deputy Director
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Abstract Title: The CEHS Administrative Core

Author(s): John M. Essigmann, Bevin P. Engelward, Sophea Chan Diaz, Kimberly Bond Schaefer, Amanda Tat

Abstract:

The mission of the Massachusetts Institute of Technology (MIT) Center for Environmental Health Sciences (CEHS) is to study the biological effects and processes of exposure to environmental agents in order to understand and predict how such exposures affect human health and the health of the ecosystem upon which people depend.

Environmental Health Sciences research at MIT encompasses a very wide range of disciplines. The Center brings together forty-three MIT faculty and research members from a total of nine MIT departments (in both the School of Science and the School of Engineering), plus one Harvard faculty member from the Harvard School of Public Health (HSPH).

The Administrative Core of the Center under the leadership of Professors John M. Essigmann and Bevin P. Engelward with input from both the CEHS Internal and External Advisory Committees are responsible for the overall scientific direction of the Center. The Center’s leadership is supported by the Administrative Officer who is responsible for the day-to-day management of the Center as well as fiscal oversight of the Center funding. The Administrative Core headquarters office consists of Amanda Tat (Administrative Officer), Sophea Chan Diaz (Fiscal Officer), Kimberly Bond Schaefer (Senior Administrative Assistant), and an Event Coordinator. In addition to the Administrative Core, the Center has a Community Outreach Education and Engagement Core (COE2C) directed by Dr. Kathleen M. Vandiver. The COE2C focuses on educational activities for health care professionals, for the K-12 school community and for their families, and manages community interactions with the Environmental Justice community in Chelsea, MA (please visit the COE2C abstract/poster).

The Center supports four Facilities Cores (please visit their abstracts/posters). The services of these Cores are available to Center members to aid in their research endeavors. The online inquiry form can be found on the CEHS website http://cehs.mit.edu/core-facilities/facilities-core-inquiry-form.

The CEHS Facilities Cores are:

1) Animal Models Facilities Core
2) Bioanalytical Facilities Core
3) Genomics and Imaging Facilities Core
4) Integrative Health Sciences Facilities Core

The Administrative Core provides its members with grants management support with regard to both pre and post award activities. The Administrative Core serves the CEHS community by supporting various enrichment activities, such as Friday Forum Seminars, BATS (Bioengineering and Toxicology) Seminars, DRAM (DNA Repair and Mutagenesis) Seminars, Newsletters, the Annual Poster Session, etc. It also manages the Pilot Project Program, which aims to attract new ideas and people to the CEHS.
CEHS Lab Affiliation: CEHS Core

Poster Presented by: Kathleen M. Vandiver

Abstract Title: The CEHS Community Outreach Education and Engagement Core (COE2C) in Action in Four Community Projects

Abstract: Kathleen M. Vandiver

Environmental Health Science Core Centers around the country use the term “community” to refer to either a geographic location or to a subgroup of the general population. At MIT we use both definitions. On this poster, we highlight MIT COE2C’s activities in four communities. 1) To begin, our first community has a geographic focus that encompasses the environmental justice (EJ) communities in Malden, MA. Malden’s population is 25% recent immigrant Chinese. Within the city there is a river called the Malden River and this waterway is fenced off due to industrial pollution concerns. This past year, COE2C worked with the Chinese Culture Connection. We asked community members how they would utilize a greenway space along the Malden River were it to be available for public use. This input was provided to the Mass Department of Environmental Protection at a public forum by COE2C director Vandiver. Thanks in part to EJ community enthusiasm in regard to the proposed greenway space, progress is being made. MIT is also assisting in the human health risk study that is being undertaken for this project. 2) Secondly, the MIT COE2C has been working with the Passamaquoddy tribe in northern Maine responding to their concerns. For example, the center participated in the EPA Region 1 Tribal Summit, visited tribal lands to collect soil samples for heavy metal analysis, and hosted tribal members at MIT for training on CEHS analytical equipment. 3) Our third community is also a partnership with an indigenous community, but located much further away. The Kohala Center is located on the big Island of Hawaii and this organization is dedicated to improving the lives of Hawaiian natives. CEHS faculty member Jesse Kroll was awarded an EPA STAR grant to create a sensor network on the island. The sensors will be placed in Kohala Garden Schools and COE2C will lead teacher workshops at these schools. Air quality in Hawaii can be poor due to volcanic emissions. 4) And lastly, MIT COE2C has chosen ‘educators’ as a community we can serve. COE2C has made great strides in responding to teachers’ needs for environmental health literacy tools. This year we are disseminating innovative molecular biology sets from MIT for teaching about genes and environment. In conclusion, these four communities are major beneficiaries of the NIEHS Core Centers program.
CEHS Lab Affiliation: CEHS Core
Poster Presented by: James G. Fox

Abstract Title: The CEHS Animal Models Facilities Core
Author(s): James G. Fox

Abstract:

Animals continue to be important models for the study of molecular mechanisms of complex biological processes related to environmental exposures. The AMC provides and maintains genetically engineered mice that are increasingly being generated to model specific aspects of human diseases and have proven to be extremely valuable in examining how genetic alterations interact with environmental chemicals and microorganisms to induce disease. Having personnel with this expertise as well as centralized laboratory equipment will provide critically important resources for the CEHS. The overall objective of the Animal Models Core (AMC) is to provide Center members with state-of-the-art pathology support, transgenic resources and a centrally managed AAALAC-accredited animal resource and surgical facility. The Core is staffed with experienced personnel and is equipped with essential equipment to generate genetically engineered mice, rederive imported mice by embryo transfer rederivation, provide colony management, utilize gnotobiotic technology, develop surgical models and prepare and interpret tissue samples by histological and immunohistochemistry analysis. The CEHS will continue to rely heavily on animal models, particularly genetically engineered mice (GEM). The models being used by Center members will require extensive characterization of their tissue phenotypes. Both the technical aspects of obtaining excellent quality sections and expertise in the identification of pathological changes are essential for the discovery of meaningful genetic and epigenetic changes that are responsible for the development of lesions. Unfortunately, this histopathologic expertise is not always available to biomedical research groups and, when absent, can lead to the misinterpretation of data. Six boarded DVMs and 2 board-certified veterinary pathologists and a fully staffed diagnostic and histology laboratory provide essential transgenic, embryo transfer derivation, colony management, germ free technology, histology, pathology and clinopathologic services that will enhance the research programs of Center members.

DCM operates approximately 200,000 sq. ft. of centrally managed AAALAC approved facilities with the necessary equipment, space and containment suites to conduct transgenic and germ-free work. The Division operates a centralized transgenic facility for generation of novel genetically-engineered rodents. The unit employs 4 FTE’s who maintain appropriate donor and recipient mouse colonies and perform microinjection of zygotes and blastocysts to produce transgenic and knockout mutant mice. A four room transgenic suite of 819 sq. ft. is located in Building 46.

The clinical and research laboratory area consists of a 720 sq. ft. virology/serology laboratory, a 335 sq. ft. necropsy suite, a 720 sq. ft. diagnostic laboratory, a 461 sq. ft. histology lab, and a glassware/autoclave room of 355 sq. ft. The DCM surgical facilities are located in the animal resource unit at Whitaker College of Health, Sciences, Technology and Management (Building E25) and in the McGovern Institute for Brain Research (Neuroscience Center, Building 46).
CEHS Lab Affiliation: CEHS Core

Poster Presented by: Pete Wishnok, Koli Taghizadeh

Abstract Title: The CEHS Bioanalytical Facilities Core

Author(s): Pete Wishnok, Koli Taghizadeh

Abstract:

The Bioanalytical Laboratories (MIT Building 16-720) provide a comprehensive and versatile set of equipment and expertise for use by Center Members. The main component consists of the dedicated Center Core laboratories, under the supervision of Dr. Taghizadeh in consultation with Dr. Wishnok.

Education and training is a high priority of the Core, and analyses are more often carried out after appropriate training, by the researchers themselves rather than by Core personnel, although there are typically experienced postdoctoral researchers or graduate students associated with Center Members’ research groups who are available for consultation and assistance.

Major equipment includes gas chromatograph-mass spectrometers (GC-MS), liquid-chromatograph mass spectrometers (LC-MS): single-quadrupole, tandem quadrupole, ion-trap (Orbitrap), and quadrupole-time-of-flight), and an inductively-coupled-plasma mass spectrometer (ICP-MS), along with an extensive collection of free-standing liquid chromatographs with most types of detectors. Bioanalytical software, in addition to the instrument control/data analysis routines, includes statistics, protein database searching, metabolite identification, and biochemical pathway analysis packages.
Abstract Title: The CEHS Genomics and Imaging Facilities Core: Instrumentation available in the Imaging Facility

Author(s): Robert Croy

Abstract:

The CEHS Imaging Facility is part of the Genomics and Imaging Facilities Core that provides imaging support for the diverse experimental strategies utilized by Center members. The Core contains instrumentation to obtain both high-quality images of tissues and cells, as well as perform high-throughput screening. Computer infrastructure and integrated facilities for high-throughput image acquisition and analysis are also available. Instrumentation includes: The Metasystems Imaging System that is equipped with a slide holder/feeder that allows for the rapid scanning of up to 80 slides. Its auto-focus feature automatically determines the correct focal plane for each slide independently. The system is highly adaptable, with the potential to define/train the system to identify any feature of interest. The Cellomics ArrayScan HCS Reader is a completely automated, wide-field microscope with 6-color fluorescent capabilities, and the capacity to scan 96-well plates. The Cellomics system is capable of low-magnification (2x) up to high magnification (40x) imaging. Further, the system has the vHCS Discovery ToolBox Software package to analyze and visualize the large datasets. We have also purchased a number of Bioapplication software packages that provide optimized parameters for analysis of cell morphometric and biochemical features. Flow cytometry analysis is also available with the acquisition of the Accuri C6 Flow Cytometer. This benchtop instrument provides Center members with a convenient and flexible system for analysis of cell cycle, gene expression and immunophenotyping of cells. It has capabilities for detecting widely used fluorochromes and performing light scattering analyses. Software included with this instrument controls data collection and permits multiparameter statistical analyses. The Accuri C6 can also be used for bead-based immunoassays. The Typhoon FLA 700 Bioimaging System in 2012 provides a versatile system for quantitative analysis of phosphorescent, chemiluminescent and colorimetric samples. CEHS members use the Typhoon for quantitative phosphoimage analysis for radioactive samples and analyses of western blots. All Imaging Facility instrumentation can be reserved through a Google calendar sign-up that is available to trained users.
CEHS Lab Affiliation: CEHS Core

Poster Presented by: Stuart Levine

Abstract Title: The CEHS Genomics and Imaging Facilities Core: Genomics

Author(s): Jon Penterman, Austin Hendricks, Noelani Kamelamela, Fangming Zheng, Stuart Levine

Abstract:

Stop by and learn about what is new in the Genomics arm of the CEHS Genomics and Imaging Facilities Core.
CEHS Lab Affiliation: CEHS Core

Poster Presented by: Stuart Levine

Abstract Title: The CEHS Genomics and Imaging Facilities Core: BioInformatics

Author(s): Vincent Butty, Duan Ma, Huiming Ding, Jingzhi Zhu, Stuart Levine

Abstract:

Come learn about what is new in the Bioinformatics arm of the CEHS Genomics and Imaging Facilities Core.
Abstract Title: Estimating Population Size from Sewage Samples with Microbial Community Data

Author(s): Fangqiong Ling, Mariana G. Matus, Ilana Brito, Claire Duvallet, Elizabeth B. Kujawinski, Eric J. Alm

Abstract:

Urban sewage represents a resource of invaluable human health data, but in order to tap this data source, we require the ability to accurately interpret the variation of biological and chemical signals in sewage, which is confounded by the variation of the target loading itself and the fluctuation in the population that contribute to the sample. As a source of public health data, sewage surveillance is most uniquely well-suited when passive sample collection, longitudinal sampling, and the incorporation of urban geographic information are needed, such as the tracking of infectious diseases, illicit drug usage, and community health indicators. The population size needs to be estimated in near real time, where traditional population estimation methods like population census would not apply. We have developed a model based on the composition of human gut microbial communities to estimate the population size in a mixed sample. This model, when coupled with an appropriate sewage sampling scheme that captures sewage before human microbial biomass can degrade, can be used for near real-time population estimation. The model also sheds light on population estimation for other aggregate samples from the built environment microbiome.
CEHS Lab Affiliation: Alm

Poster Presented by: Fangqiong Ling

Abstract Title: Microbial Community Assembly in Building Water Supplies

Author(s): Fangqiong Ling, Mark W LeChevallier, Rachel W. Whitaker, Wen-Tso Liu

Abstract:

Potable water supply systems harbor diverse microorganisms that humans are exposed to through drinking, inhalation, and skin contact. Various factors in water production and distribution have been shown to shape the microbial communities in city tap water, however, the effects of water supply pipes in buildings have not been directly tested. Here we examine the tap water bacterial community change in buildings where water use was strictly controlled, and show that community compositions drastically different from fresh city tap assembled in building water supplies when water use was paused. The community compositions correlated to the physical structure of the water supply pipes and were highly consistent across different buildings, floors, and faucets. The communities assembled in the same faucet was resilient to disturbance. Water from individual faucets could be modeled under a neutral community assembly framework as islands connected to two mainlands – city tap water and distribution system biofilms. The stagnant water communities in proximal pipes were mainly driven by tap water sources, while those in distal pipes were driven by biofilms. Our results suggest that the physical structure of water supplies dictates the community assembly in building water supplies in predictable processes, enable design of water microbiome.
Abstract Title: Bacillus Cell Growth Processes Investigated Through Live, Single-Molecule Imaging

Author(s): Zachary Barry, Ethan Garner, Mark Bathe

Abstract:

The visualization of single molecules within a living cell is informative as to their roles within a larger, systems-biological context. These molecules undergo a variety of motions, including pure diffusion and directed motion or transport. Motion is directly tied to molecule function, their interacting partners, and changes in activity they undergo as conditions change within the cell.

Here, we observe the machinery that manufactures the cell wall, the structure that confers cell shape and stability in Bacillus subtilis. As a case study, single molecules of a filament (MreB) and enzyme (PbpA) both implicated in this process are visualized through total internal reflection (TIRF) microscopy. These molecules have been discovered to undergo heterogeneous, switching dynamics where they exhibit not only predominantly directed motion during the synthesis of cell wall but, in addition, pauses, reversals in direction, and switches between diffusion and directed motion. We approach the problem of analysis of these single-molecule motions and the reasons behind them through the application of single-particle tracking and statistical motion analysis (via hidden Markov modeling) techniques.
Abstract Title: **TRM9L is a Component Of The ERK Pathway in Response to H2O2 and Phosphorylation is Crucial for its Tumor Suppressive Function**

Author(s): Chen Gu, Ulrike Begley, Thomas J. Begley, Peter C. Dedon

Abstract:

Due to alterations of central metabolism and development of a tumor microenvironment, cancer cells often suffer a high degree of oxidative stress. Many antineoplastic therapies thus work by inducing a storm of ROS to tip the delicate intracellular redox balance maintained in cancer cells. In addition, oxidative stress often serves as a barrier that cancer cells must overcome for successful tumorigenesis and metastasis. Previously, we showed that the human TRM9-like protein (TRM9L), a homolog of the S. cerevisiae tRNA methyltransferase 9 (TRM9), has tumor growth suppressive properties. Here we demonstrate that one phenotype of TRM9L expression is hypersensitivity to the oxidative stress of H2O2 exposure but not to oxidative stress of γ-radiation (γ-Rad), a relatively pure source of hydroxyl radicals. We identified hTRM9L as a phosphoprotein and its phosphorylation status depended highly on the extracellular environment. For example, trypsinization during processing of monolayer cells led to rapid phosphorylation of hTRM9L, with some phosphorylation sites mapped to Ser 214, Ser 255, Ser 291, and Ser 380. Among these sites, H2O2 increased phosphorylation at Ser 255, Ser 291, and Ser 380 but not Ser 214. In particular, the ribosomal s6 kinase (RSK), upon phosphorylation by H2O2-activated extracellular signal-regulated kinase (ERK), phosphorylated the Ser 380 site. On the other hand, γ-Rad did not have a significant effect on the phosphorylation status of Ser 380. Relative to the wild-type, the TRM9L S214A and the S255A mutants showed significantly reduced tumor growth suppression, indicating that phosphorylation is crucial for the tumor suppressive functions of this protein. Modeling suggested that the TRM9L domain on which all 4 phospho-residues are located is intrinsically disordered, based on which we hypothesize that phosphorylation mediated by ERK-RSK and other potential kinases confers rigidity to the structure of this domain and hence activates the protein for tumor growth suppression.
Abstract Title: Abasic Site Cross-link Discovery and Quantification via stepped MRM in Rat Tissue. A Novel Approach to Identify DNA Modifications in Organisms

Author(s): Roman Hillebrand, Michael J. Catalano, Sushovan Mohapatra, Samuel Senyo, Richard Lee, Kent S. Gates, Peter C. Dedon

Abstract:

The Dedon group has developed a new mass spectrometry method for discovering novel DNA and RNA damage products and modifications, which we term “stepped MRM”. The method combines the sensitivity of multiple reaction monitoring (MRM) with the discovery power of MS scanning to systematically search, in 1 Da increments over a 30 m/z window from 200 – 410 m/z, for unknown 2-deoxynucleoside structures in enzymatic hydrolysates of DNA. Applying this approach to DNA isolated from rats we found a variety of previously undescribed DNA damage products in different tissues. The structures of these novel 2-deoxyribonucleosides are currently being established. Stepped MRM also revealed age-dependent increases in a previously described adduct, N2-carboxymethyl-dG (CMdG), in liver. CMdG is known to arise from reactions of DNA with glyoxal, the ubiquitous dialdehyde derived from oxidation of glucose, protein and lipids, and involved in formation of advanced glycation end-products associated with chronic disease. The stepped MRM approach thus provides a means to identify the DNA damage products and modifications that truly matter in normal development and tissue-specific pathophysiology of aging. An especially interesting discovery is a dA-Ap interstrand crosslink. The loss of a coding nucleobase from the structure of DNA is a common event (50,000 – 200,000/cell) that generates an abasic (Ap) site. Unrepaired abasic sites are mutagenic or cytotoxic. These Ap sites can react with dA on the opposite strand to form interstrand crosslinks. The Dedon lab is collaborating with researchers in Prof. Richard Lee’s group at the Harvard Medical School and Massachusetts General Hospital and Prof. Kent S. Gates’ group at the University of Missouri - Columbia to quantify these dA-Ap sites and establish age-dependent changes in different tissues taken from rats aged 1 day, 4 months, 1 year and 3 years.
Abstract Title: Structural Characterization of a Hydroxyproline Dehydratase from C. difficile

Author(s): Lindsey Backman, Yolanda Huang, Michael Funk, Emily P. Balskus, Catherine L. Drennan

Abstract:

The glycyl radical enzyme (GRE) family utilizes a glycyl radical cofactor in order to catalyze difficult chemical reactions in a variety of microbial pathways, including glycolysis, DNA synthesis, and in the metabolism of important biomolecules such as toluene, choline, tyrosine, and glycerol. Although glycyl radical enzymes are widely encoded and expressed by bacteria found in the gut microbiome, these enzymes remain largely uncharacterized. Recently, a new glycyl radical enzyme was discovered to catalyze the dehydration of trans-4-hydroxy-L-proline to 1-pyrroline-5-carboxylic acid, a metabolic intermediate in fermentation and amino acid biosynthesis. Bioinformatics studies by the Balskus lab show that this hydroxyproline dehydratase (HPDH) is encoded by 360 bacterial genomes, many of which are associated with the human gut and oral cavities, including the human pathogens C. difficile, C. botulinum, and T. maltophilum. Their studies indicate that this uncharacterized enzyme is one of the most abundant glycyl radical enzymes found in the human gut, second only to pyruvate formate-lyase. Many bacterial species which express HPDH are known to use amino acid electron donors and acceptors as their method of primary metabolism, referred to as Stickland fermentation, and studies have shown that hydroxyproline can be utilized as an electron acceptor through an unknown pathway. HPDH could be the missing puzzle piece as to how these bacterial species use the abundant metabolite hydroxyproline as a source of energy. In order to elucidate the mechanism for how HPDH performs the dehydration of hydroxyproline, we aimed to characterize HPDH from C. difficile, in the presence of its substrate, via X-ray crystallography. Here, we have solved a 2.05-Å resolution structure for HPDH by molecular replacement, using the characterized GRE CutC as the starting model. Subsequently, structures for HPDH with trans-4-hydroxy-L-proline bound in the active site were solved at resolutions ranging from 2.60 to 3.20-Å resolution.
Abstract:
In all organisms, ribonucleotide reductase (RNR) catalyzes the precisely-balanced irreversible conversion of ribonucleotides into deoxyribonucleotides for DNA synthesis and repair. In human RNR, changes in oligomerization of α, the RNR large subunit, accompany modulation of enzyme activity in response to changing concentrations of nucleotide effectors and appear critical for clinical inhibitors. To understand the mechanisms of allosteric regulation, we used single particle electron microscopy (EM) to examine the human RNR α subunit under conditions where it is inhibited and assembles into an α6 oligomer. Our ~3.3 Å resolution cryo-EM reconstruction shows that inhibition results from specific interactions between N-terminal cone domains of α that stabilize rigid α6 rings and preclude assembly of a catalytically competent complex with β2, the RNR small subunit, without perturbing conserved aspects of allosteric substrate selectivity.
CEHS Lab Affiliation: Drennan

Poster Presented by: Tsehai A.J. Grell

Abstract Title: Structural Investigation of an AdoMet Radical Enzyme Involved in RiPP Biosynthesis

Author(s): Tsehai A.J. Grell, Nathan A. Bruender, Vahe Bandarian, Catherine L. Drennan

Abstract:

With an increasing number of drug resistant bacteria, the search for new antibiotics is of utmost importance. Members of the newly identified class of natural products, Ribosomally synthesized and post-translationally modified (RiPPs), display a wide variety of structures and bioactivities. Therefore understanding the biosynthesis of these natural products could provide an untapped source of inspiration for new drug compounds.

Sactipeptides are a subclass of RiPPs, which show antimicrobial properties. They are characterized by the presence of a head-to-tail macrocylic ring and one or more unusual intramolecular thioether bond between the sulphur of a cysteine residue and the -carbon of an acceptor amino acid. The installation of the unusual thioether bond is the first step in the maturation of sactipeptides and is performed by members of the SPASM subclass of the S-Adenosylmethionine (AdoMet) radical enzyme superfamily. The AdoMet radical enzymes utilizes the direct ligation of a molecule of AdoMet to a reduced [4Fe-4S] cluster to produce a 5'-deoxyadenosyl radical (5'-dAdo•) species, which is able to abstract a hydrogen atom from the acceptor residue of the thioether bond, initiating thioether bond formation. In order to elucidate the mechanism by which the AdoMet radical enzymes facilitate the thioether bond formations, SkfB, an enzyme in the maturation of the sactipeptide sporulation killing factor (SKF) was structurally characterized. Here report a structure of the Holo-SkfB in complex with it’s co-substrate AdoMet to 1.28 Å-resolution. This structure represents the first of the AdoMet radical enzymes that are involved in RiPP biosynthesis. From this structure we can begin to understand how the precursor peptide substrate, SkfA, may bind to SkfB, with its leader peptide guiding the way as well as hypothesize about the structure of other members of this class. With this information, we could begin engineer the sactipeptide scaffold to tune it specific biological activities.
CEHS Lab Affiliation: Drennan

Poster Presented by: Gyung Hoon Kang

Abstract Title: Probing the Active State of Ribonucleotide Reductase Using Electron Microscopy

Author(s): Gyung Hoon Kang, Edward J. Brignole, Alex Taguchi, Francisco J. Asturias, JoAnne Stubbe, Catherine L. Drennan

Abstract:

Ribonucleotide reductase (RNR) is the only enzyme in all organisms responsible for the conversion of ribonucleotides to deoxyribonucleotides, and as such, plays a critical role in both DNA biosynthesis and repair. Remarkably, all RNRs are capable of reducing all four ribonucleotide substrates, and are therefore not only responsible for maintaining the proper level of deoxyribonucleotides overall, but must also be able to balance the ratio between them. Given RNR's crucial role in biology, it has been the target of ongoing drug development for anti-cancer, anti-viral, and anti-parasitic therapies.

E. coli has been the prototypic organism when it comes to studying RNR. Through decades of biochemical work, we now understand much about how RNR works. Yet despite our best efforts, a high-resolution structure of the active state of RNR has been elusive, mainly owing to the weak interaction between the two subunits that are required to come together for activity. Here, we present our current efforts to probe the active state of E. coli RNR using electron microscopy.
CEHS Lab Affiliation: Engelward
Poster Presented by: Christy Chao

Abstract Title: Biological Impacts of Lanthanide Exposure
Author(s): Christy Chao, Stephen Slocum, Clint Valentine, Bevin Engelward, John Essigmann, Harold Hemond

Abstract:

The lanthanides are a group of fifteen elements that include lanthanum (Z=57) through lutetium (Z=71). They are part of the family of metals known as the rare earth elements. Lanthanide usage has increased in recent years due to numerous applications in devices such as TVs, cars, or satellites. They are used in the medical field as imaging agents, as fertilizers in the agriculture industry, or as fuel additives in the petroleum industry. Usage is expected to grow, however little is currently known about the behavior of these metals in biological systems and the health risks they pose. While metals such as mercury or lead have been heavily studied and exposure effects are clearly understood, lanthanides have not been researched to the same degree. Some toxicological studies have been performed for the lanthanides, however there remains a significant knowledge gap in the behavior of these metals. We propose that these compounds can induce varying levels of damage to cells and DNA, and can potentially impact a cell’s repair capacity. We also propose that exposure to lanthanides can impact a cell’s vulnerability to reactive oxygen species. Using cellular systems, the cytotoxicity and genotoxicity of lanthanide chlorides and lanthanide oxide nanoparticles are being assessed as part of the effort to learn about the health risks associated with exposure to these compounds. The extent to which different DNA repair pathways and proteins are involved in repairing lanthanide-induced damage will also be examined. Understanding the genotoxic potential and negative health impacts of these materials is a necessary step in learning how to protect against lanthanide-induced damage in those who are most at risk.
CEHS Lab Affiliation: Engelward

Poster Presented by: Jennifer Kay

Abstract Title: Exploring the Contributions of Inflammation and DNA Damage to Carcinogenic Mutations

Author(s): Jennifer Kay, Orsolya Kiraly, Susan Erdman, Bevin Engelward

Abstract:

Cancer is a disease of genetic mutations resulting in unchecked cell growth and metastasis. Mutations are therefore extremely important to study, but difficult to query due to laborious, expensive assays that are limited in scope, resolution and detail. In order to study a broad spectrum of mutations, our lab has developed the RaDR Recombomouse, which allows detection of cells that have undergone mutagenic double strand break (DSB) repair via homologous recombination (HR). This transgenic marker system, comprised of two truncated copies of Egfp, produces a full-length sequence if there is a misalignment during HR, and therefore a fluorescent cell. Because the genetic rearrangement leading to a fluorescent cell is inherited by daughter cells, we are also able to visualize the progeny of the original recombined cell, providing insight into clonal expansion. These mice have also been bred to include the Gpt-Δ/Spi transgene (mice developed and donated by Takehiko Nohmi) for detection of point mutations and deletions, enabling an unprecedented breadth and depth of mutational analysis.

To expand our understanding of the origins of carcinogenic mutations, we are now studying inflammation and DNA damage in an animal model of colorectal cancer. ApcMin/+ mice were treated with DSS to mimic colitis and/or AOM to represent many of the numerous damage-inducing chemicals we encounter daily. Our studies include mutational assays within the intestines as well as across other tissues, potentially revealing distal effects of localized damage. Results will provide high-resolution analyses of mutagenesis and carcinogenesis resulting from inflammation and alkylation damage, carcinogenic exposures encountered by virtually all humans.
Abstract Title: Novel Microarray Colony Formation Platform for High-Throughput Viability Testing

Author(s): Le Ngo, Tze Khee Chan, Jing Ge, Leona Samson, Bevin Engelward

Abstract:

Quantification of cell survival is one of the most fundamental and broadly used endpoints in biology. There are ~1000 new chemical compounds introduced each year, for which toxicity testing is much needed. Survival assays are also a mainstay assay in the pharmaceutical industry where they are used to predict adverse effects as well as establishing efficacy of compounds designed to target cancer cells. In these contexts and others, accurate cell survival testing has significant implications. The gold standard for cell survival quantification is the colony forming assay, wherein cells are exposed to a toxic agent and the ability of single cells to subsequently form colonies is quantified. While the assay has an impressive dynamic range over several orders of magnitude, it is relatively low-throughput (10-21 days), laborious, and prone to bias due to manual colony counting. To overcome some of the significant shortcomings of the colony forming assay, high-throughput assays have been developed wherein indirect measures of cell viability are used to estimate the extent of cytotoxicity. Currently, the most commonly used assays for cytotoxicity rely upon membrane integrity (e.g. trypan blue exclusion) and metabolic activity (e.g. MTT/XTT, CellTiter-Glo®) as endpoints. Although popular, some of these assays suffer from low sensitivity and an inferior dynamic range of measurement compared to the colony forming assay. Importantly, membrane integrity and metabolic activity do not always correlate directly with viability and are susceptible to viability-independent interferences, such as changes in pH, factors that affect cellular metabolism, and constituents in cell media (e.g. reducing agents).

Here we describe MicroColonyChip (µCC), a novel microarray colony formation platform for cell viability testing that overcomes the major limitations of existing assays. The microarrayed colony format enables the miniaturization of the traditional colony forming assay, representing a paradigm shift where the portion of surviving cells is measured via total DNA content in a high-throughput manner. Toxicity is calculated based on the change in the distribution of the microcolony sizes over a period of a few days in culture. Rather than counting colonies by eye, the sizes of the microcolonies can be estimated in an automated fashion by using a DNA stain. Being able to grow cells in a microarray enables ~250 fold increase in colony density, which makes it possible to move from large dishes to a 96 well plate. In addition, automated imaging and image analysis enables high throughput quantification of colony size. As a result, the µCC assay is much faster than the traditional colony forming assay (up to five days), and requires very little media and test compound. We have developed methods to analyze colony size distribution to extract key parameters reflecting cell viability. These variables form the basis of a highly sensitive toxicity test. Here, we show that the µCC has sensitivity comparable to the colony forming assay. We also show that the µCC is more sensitive than the XTT assay, and that it has comparable sensitivity to CTG®, while being easier to execute. Finally, we show that the µCC can be used for studies of xenobiotics in metabolically relevant conditions. Taken together, µCC offers the direct measure of a cell’s ability to divide, similar to the colony forming assay, and the scale and speed of microtiter assays, such as the XTT and CTG® assays, thus offering a generally useful toxicity
Abstract Title: The CometChip: A Tool for Epidemiological Studies and High Throughput Screening

Author(s): Ian J. Tay, Jing Ge, Le Ngo, Scott R. Floyd, Bevin P. Engelward

Abstract:

DNA repair is a critical process in maintaining genomic integrity in cells. When this process is faulty in healthy cells, cells will accumulate mutations in their DNA, which can lead to cancer and other diseases. Conversely, for cells which are already cancerous, the ability of those cells to repair DNA modulates the effectiveness of radio- and chemotherapies that target DNA in order to kill them. Understanding the range of DNA repair capacities in apparently healthy cells as well as the underlying genetic mechanisms that modulate this repair process will enable us identify vulnerable members of the population and lead to new targets to enhance existing therapy regimens for cancer.

We have previously developed the CometChip, a novel adaptation of the traditional comet assay that can be used to measure DNA damage in cells. The CometChip leverages micron-scale patterning of cells in agarose with spatial consistency, which enables a vast increase in throughput and sensitivity of the assay. It also allows comets to be automatically imaged and analyzed. The increase in throughput of the assay allows multiple conditions to be tested on a single run of the assay, thus enabling us to perform DNA damage and repair experiments at a scale that was not possible before.

In the first part of this work, we have tested a panel of 22 lymphoblastoid cell lines derived from healthy individuals, for their ability to repair alkylation and oxidation induced DNA damage. We have observed a lower level of variation in DNA damage levels when an alkylating agent was used, compared with an oxidizing agent. We have also observed two distinct groups of cell lines that produce a relative constant signal level over time in our assay when they were challenged with an oxidizing agent, suggesting that one group of cell lines may be more susceptible to oxidative DNA damage than the other. Moving forward, we will be investigating possible differences between the two groups of cell lines, such as the level of activity of specific enzymes that can explain the phenotype that we observed.

In the second part of this work, we have also further improved on the design of the CometChip, such that it is now compatible with HTS robotics. We have utilized the HTS CometChip to screen 7,500 shRNAs, representing 2,500 genes in a lentiviral shRNA gene library that target oncology related genes. The genes were preliminarily ranked according to their impact on DNA repair. In recent experiments, we discovered that some genes may have an effect on apoptosis or cell death, independently of the levels of DNA damage in cells, and might be represented in our list of candidate genes. We will be analyzing the images from the screen in greater detail to further characterize this observation and further study genes that impact DNA repair or apoptosis.
CEHS Lab Affiliation: Essigmann

Poster Presented by: Jake Campolo

Abstract Title: Evaluating the Therapeutic Potential of 11β Compounds in a Novel, Cell Culture Model of Polycystic Kidney Disease

Author(s): Jake Campolo, Sakunchai Khumsubdee, Robert G. Croy, John M. Essigmann, Bogdan I. Fedeles

Abstract:

Polycystic kidney disease (PKD) is a monogenic genetic characterized by the formation and growth of fluid-filled cysts in the kidneys. Significant enlargement of the kidneys leads to impaired function and complications such as pain, high blood pressure, and eventual kidney failure. Although it affects up to one in 400 people globally, there is currently no FDA-approved drug to treat PKD. Originally developed by the Essigmann lab to treat prostate cancer, the antitumor agent 11β-dichloro and its derivatives have been demonstrated to drastically reduce the size and cystic index of PKD-afflicted kidneys in a mouse model of PKD by preferentially triggering apoptosis in cyst cells through mitochondrial disruption and induction of oxidative stress. In this study, we developed a cell culture model for studying 11β compounds and their therapeutic potential for PKD treatment. Three isogenic pig kidney epithelial cell lines (derived from LLC-PK1) were cultured: PKD1 and PKD2 knockout (causative genes for PKD), as well as the parental wild type cell line. These cells were treated with 11β compounds over a range of concentrations, and analyzed with several cell viability assays to determine the most consistent and effective protocol to measure the compounds' efficacy. We investigated the baseline differences between each genotype, such as mitochondrial number and transcript levels of reactive oxygen species (ROS)-modulating genes, in order to gain more insight into the mechanism of action and the compounds' selectivity for cystic cells. A reliable cell culture model and a greater understanding of 11β’s mechanism will further its development as a drug candidate for treating PKD.
CEHS Lab Affiliation: Essigmann

Poster Presented by: Supawadee Chawanthayatham

Abstract Title: Mutational Spectra of Aflatoxin B1 in Vivo Establish Biomarkers of Exposure for Human Hepatocellular Carcinoma

Author(s): Supawadee Chawanthayatham, Bogdan I. Fedeles, Charles C. Valentine III, Edward J. Fox, Lawrence A. Loeb, Stuart S. Levine, Stephen L. Slocum, Gerald N. Wogan, Robert G. Croy, John M. Essigmann

Abstract:

Aflatoxin B1 (AFB1) and/or hepatitis B and C viruses are risk factors for human hepatocellular carcinoma. Available evidence supports the interpretation that formation of AFB1-DNA adducts in hepatocytes seeds a population of mutations, mainly G:C→T:A, and viral processes synergize to accelerate tumorigenesis, perhaps via inflammation. Responding to a need for early-onset evidence predicting disease development, highly accurate duplex sequencing was employed to monitor acquisition of high-resolution mutational spectra (HRMS) during the process of hepatocarcinogenesis. Four-day old male mice were treated with AFB1 using a regimen that induced hepatocellular carcinoma within 72 weeks. For analysis, livers were separated into tumor and adjacent cellular fractions. HRMS of cells surrounding the tumors revealed predominantly G:C→T:A mutations characteristic of AFB1 exposure. Importantly, 25% of all mutations were G→T in one trinucleotide context (CGC), which is also a hotspot mutation in human liver tumors whose incidence correlates with AFB1 exposure. The technology proved sufficiently sensitive that the same distinctive spectrum was detected as early as 10 weeks after dosing, well before evidence of neoplasia. Additionally, analysis of tumor tissue revealed a more complex pattern than was observed in surrounding hepatocytes; tumor HRMS were a composite of the 10-week spectrum and a more heterogeneous set of mutations that emerged during tumor outgrowth. We propose that the 10-week HRMS reflects a short-term mutational response to AFB1, and as such is an early detection metric for AFB1-induced liver cancer in this mouse model that will be a useful tool to reconstruct the molecular etiology of human hepatocarcinogenesis.
Abstract Title: The Mutagenic Signature of 5-Chlorocytosine and the Connection between Inflammation and Cancer

Author(s): Tania J. Gonzalez-Robles, John Essigmann, Bogdan Fedeles

Abstract:

Genomic DNA is under constant biochemical pressure due to detrimental exogenous, as well as endogenous DNA damaging agents. One example of an endogenous burden on DNA is inflammation. It is known that persistent inflammation can promote and exacerbate a malignancy; chronic colon inflammation (colitis) leading to colon cancer and chronic viral infections leading to liver cancer are well-documented examples. During inflammation, white blood cells, including neutrophil granulocytes, infiltrate the injured tissue, and, as part of the immune system’s efforts to protect the host from foreign pathogens and infection, they release a plethora of reactive oxygen, nitrogen and halogen species, such as hydrogen peroxide, nitric oxide and hypochlorous acid. The neutrophil-derived hypochlorous acid, commonly known as bleach, results from the reaction between hydrogen peroxide and chloride, a reaction catalyzed by the primary granule protein called myeloperoxidase. The hypochlorous acid can react directly with many biomolecules, including genomic DNA, where it forms the 5-chlorocytosine (5ClC) DNA adduct. The biological relevance of 5ClC and its inflammation biomarker properties have been previously established by the Dedon and Tannenbaum labs, who found accumulation of 5ClC at the sites of inflammation in a mouse model of colitis. Recently, our lab discovered that 5ClC is a mutagenic modification, causing C > T transitions when replicated in vitro and in vivo. Consequently, we are now interested in establishing the mutational signature of 5ClC, which is the relative propensity of 5ClC to induce mutations in each of the 16 possible 3-base DNA sequence contexts. For this, degenerate oligonucleotides, containing 5ClC in all possible sequence contexts are tagged with unique barcodes and cloned into M13mp7(L2) single-stranded DNA phage vectors. Following replication of the M13 phage in E. coli strains with various DNA replication and repair capabilities, the progeny phage are recovered, amplified and analyzed by next generation sequencing. Establishing the mutational signature of 5ClC is important, because it will allow us to evaluate quantitatively the amount and type of mutations that are contributed by 5ClC in inflamed tissues, and, eventually, in inflammation-related tumors.
Abstract Title: Structural Investigation of the Anti-Polycystic Kidney Disease Properties of the 11β Family of Novel, Synthetic Anti-tumor Agents

Author(s): Sakunchai Khumsubdee, Jake Campolo, Robert G. Croy, John M. Essigmann, Bogdan I. Fedeles

Abstract:

Polycystic kidney disease (PKD) is the most common genetic renal disease, which manifests by the progressive accumulation of fluid-filled cysts in the kidneys, leading to an increase in kidney volume and impaired kidney function. Many PKD patients end up requiring periodic dialysis and even kidney transplant. No FDA-approved treatments for this disease currently exist. Recently, a family of synthetic, multifunctional compounds, developed in the Essigmann lab, was demonstrated to be highly effective at preventing cystic growth in a mouse model of PKD. The compounds, such as 11β-dichloro, 11β-dimethoxy and 11β-dipropyl, were originally developed as anti-tumor agents. The compounds feature an estradieneone moiety as a ligand for cancer-specific proteins, linked to an aniline mustard (or derivative) via a non-hydrolyzable linker that contains several functional groups such as carbamates and secondary amines. Preliminary evaluation of 11β family compounds found that they rapidly induce cellular oxidative stress, which triggers apoptosis in the kidney cyst cells. However, the molecular details of the mechanism by which 11β compounds target and kill cystic cells are still unclear. A structure-activity study was setup to investigate the chemical functionalities and structures required for the pro-apoptotic properties of 11β compounds. New compounds were synthesized, featuring a variety of linkers and different substituents for the aniline mustard portion of the molecule. This poster focuses on the synthetic strategies for obtaining the different 11β derivatives, their physico-chemical properties and an overview of their biological activities.
Abstract:
The rare earth elements (REEs) consist of the crustal elements in the lanthanide series (atomic numbers 57-71), yttrium and scandium. In recent years their use has skyrocketed, thanks to their unique chemical and physical properties, in ‘next gen’ technologies including semiconductors, smart phones, permanent magnets and batteries. Indeed, production of REEs increased by ~10-fold from 1950 to 2000, with the majority of ore extraction and processing happening in China. Despite their increased extraction and use, the toxicological properties of the REEs have not been examined in depth, and the research that has been performed has yielded conflicting results. To that end, a subset of the REE metallic salts were evaluated for cell culture based toxicity. The human lung cell lines A549 (alveolar basal epithelial cells) and BEAS-2B (bronchial epithelial cells) were utilized in order to mimic the potential route of human exposure to airborne REEs. Endpoints included both cytostatic and cytotoxic effects as well as the ability of the REEs to cause reactive oxygen species (ROS) generation. Cellular toxicity may occur via independent generation of ROS, or via potentiation of H2O2 via a fenton-like reaction with REEs that have the ability to redox cycle. The cellular bioaccumulation of the REEs was evaluated by ICP-MS in order to further examine the potential mechanisms of REE toxicity. Lanthanum demonstrates mild cytostatic properties, while the other REEs monitored had little to no effect on cellular growth following exposure. REEs in concert with H2O2 showed no exacerbation of the individual REE cytostatic properties. REEs also failed to exacerbate the cytotoxic properties of H2O2 when compared to an iron control. Flow cytometry was also utilized to examine the ability of the REEs to either generate or exacerbate ROS within cells. Additional studies into the potential of REEs to synergize with environmental toxins needs to be performed to rule out the potential for cooperative toxicity. Further investigation into the REEs will help reveal the mechanisms behind any associated toxicity, and may inform public health interventions in exposed populations.
CEHS Lab Affiliation: Essigmann

Poster Presented by: Nicole Zatorski

Abstract Title: Mutagenic Consequences and BER Efficiency of Tandem 5-chlorocytosine, 8-oxoguanine DNA lesions

Author(s): Nicole Zatorski, John M. Essigmann, Bogdan I. Fedeles

Abstract:

Mutagenic consequences and BER efficiency of tandem 5-chlorocytosine, 8-oxoguanine DNA lesions DNA repair is an essential process for maintaining the integrity of genetic information; deficiencies in DNA repair are strongly linked to cancer initiation and progression. Among the different DNA repair pathways, base excision repair (BER) is one of the most versatile, repairing many kinds of oxidized, alkylated or deaminated bases. One of the most ubiquitous substrates for BER is the oxidative stress induced 8-oxoguanine (8OG), a mutagenic DNA lesion. Deficiencies in 8OG repair have been shown to lead to cancer. When 8OG is paired with C, BER is initiated by glycosylases (FPG in E. coli, hOGG1 in mammalian cells) that remove 8OG. BER is also involved in replacing the aberrant T of a T:G mismatch, which forms as a consequence of the spontaneous 5-methylcytosine (5mC) deamination, with a C. In this case, the repair is initiated by the TDG glycosylase. Recent studies have shown that the efficiency of BER is sequence-context dependent. In particular, if 8OG forms next to the deamination-induced T:G mismatch, the repair of both lesions is impaired. Recent work in the Essigmann lab uncovered the mutagenic properties of 5-chlorocytosine (5ClC), a biomarker of inflammation, and in particular neutrophil infiltration. Besides its mutagenic properties, 5ClC is isosteric with 5mC, and in fact, when it occurs in CpG sites, it can redirect the DNA methylation enzymes to introduce 5mC on the opposite strand. The current work investigated the biological consequences of 5ClC and 8OG lesions occurring in tandem, either next to each other on the same strand, or on opposite strands, as part of a base pair. We found that the presence of 5ClC modulates the efficiency of 8OG repair in vitro and consequently modulates the mutagenic properties of 8OG in vivo. Ultimately, these results may shed light on the interplay between inflammation-induced DNA damage and DNA BER, resulting in a mutagenic outcome, which could constitute a mechanistic link between inflammation and cancer.
Abstract Title: Gastric CLDN18 Loss Affects Transcellular Cl- Permeability Rather than Dysregulating Tight Junctions

Author(s): Tyler J. Caron, Nishita Sinha, Kathleen E. Scott, Lay-Hong Ang, James G. Fox, Susan J. Hagen

Abstract:

Loss of tight junction integrity is an important risk factor for H. pylori-mediated gastric cancer (GC) development. The loss of claudin (CLDN)18 in particular, a membrane spanning protein of the tight junction complex in stomach, is associated with an aggressive phenotype and poor patient outcome. The loss of CLDN18 occurs early, after the onset of intestinal metaplasia, and is thought to promote GC development. To model this outcome in mice, we recently demonstrated that CLDN18-deficient (KO) mice have severe atrophy, glandular dysplasia, and a rapid progression to intraepithelial neoplasia. CLDN18 KO mice also upregulate CLDN2, which is a leaky CLDN that increases paracellular permeability. Because the loss of CLDN18 may affect tight junction function to drive GC development, the AIM of this work was to examine the permeability of stomachs from CLDN18 KO mice using Ussing chambers. Methods: Frozen embryos from CLDN18 KO mice (B6:129S5-Cldn18tm1Lex/Mmucd) were purchased from the MMRRC and re-derived. Isolated gastric mucosa from 7 week old wild-type (WT), heterozygous (HET), or knockout (KO) mice were stripped of the muscularis externa and then mounted between two Lucite halves of an Ussing-type chamber. Tissues were maintained at 37o C with a balanced nutrient buffer on the luminal and basolateral sides and were gassed with 95% O2/ 5% CO2. Gastric acid secretion was blocked with an H2 receptor antagonist. Transepithelial resistance (TER)/conductance, potential difference (PD), the luminal to basolateral flux of FITC-labeled dextran (FD)-4, and dilution potentials were measured. Confocal microscopy was used to analyze CLDN2 expression and RNAseq was used to assess transcriptional differences between WT and KO mucosa at 7 weeks. All data were analyzed using Systat software. Results: In WT and HET mice, the PD was highly lumen negative, due to constitutive transcellular chloride flux from the basolateral to luminal surface. In contrast, the PD of tissues from KO mice was nearly zero, concomitant with a significant reduction in chloride permeability. This result occurred with no significant change in TER/conductance and no change in paracellular flux between groups. Although RNAseq indicated a large-fold increase in CLDN2 expression, confocal microscopy analysis showed substantially lower protein expression. Gene expression for the majority of known chloride channels and transporters in glandular epithelial cells was significantly down-regulated in KO mice. Conclusions: Our results suggest that there is no dysregulation of tight junction function in CLDN18 KO mice but rather, a decrease in transcellular chloride permeability. This is likely due to the lack of chloride transporters in the pre-neoplastic mucosa. We conclude that the loss of CLDN18 in GC development does not affect tight junction integrity or alter paracellular permeability per se, but instead drives neoplastic change by other mechanisms.
Abstract Title: Characterization of Multi-drug Resistant Enterococcus faecalis Isolated from Research Macaques

Author(s): Mia T. Lieberman, Stephanie E. Woods, Francois Lebreton, Cesar de la Fuente-Núñez, Joanne L. Dzink-Fox, Michael S. Gilmore, James G. Fox

Abstract:

Multi-drug resistant (MDR) Enterococcus faecalis is a common and serious cause of nosocomial infections. Previous characterization of 15 E. faecalis isolates from cephalic recording chambers of macaques used in neuroscience research revealed marked multi-drug resistance. Multi-locus sequence typing identified three sequence types among the 15 macaque E. faecalis isolates; ST 4 (n=7), ST 55 (n=7) and ST 330 (n=1). ST 4 isolates showed differing susceptibilities to gentamicin, with 4/7 isolates displaying high-level gentamicin resistance while ST 55 isolates displayed resistance to neomycin. All ST 4 and ST 55 isolates displayed marked streptomycin resistance. Resistance to tetracycline, chloramphenicol, enrofloxacin, erythromycin, bacitracin and trimethoprim-sulfamethoxazole were also noted. Following whole genome sequencing and assembly, FASTA sequences of two ST 4 isolates and one ST 55 isolate were analyzed by PubMLST, ResFinder, VirulenceFinder and PATRIC to confirm sequence type (ST) and identify genes of interest. All sequenced isolates had a unique antimicrobial resistance gene profile with the lsa(A) gene encoding intrinsic resistance to lincosamides and streptogramins A as the only common gene between all 3 isolates. Four genes encoding aminoglycoside resistance were identified: str and 3 aminoglycoside-modifying enzymes: aph(3′)-III, aac(6′)-aph(2”) and ant(6)-Ia. A variety of other antimicrobial resistance genes including erm(B), tetL, tetS, tetM, cat, bcrABD and dfrG were identified along with single amino acid polymorphisms in parC and gryA conferring fluoroquinolone resistance. A variety of biofilm-associated genes and virulence factors were identified, including the cytolsin toxin, enterococcal surface protein, aggregation substance, gelatinase, collagen adhesion precursor and endocarditis antigen. The ST 55 isolate possessed fewer antimicrobial and biofilm-associated genes than the ST 4 isolates which may be attributed to an intact type IIa CRISPR-cas system. In vitro crystal violet and flow cell assays confirmed that ST 4 isolates produced significantly more biofilm than ST 55 isolates. E. faecalis isolates from cephalically-implanted macaques display genetic similarities to isolates associated with human nosocomial infections. Macaques represent a unique research model to study nosocomial infections due to their long-term residence in a healthcare setting and intermittent antimicrobial exposure.
CEHS Lab Affiliation: Fraenkel

Poster Presented by: Tobias Ehrenberger

Abstract Title: Integrative ‘omics Data Analysis to Uncover Signaling Networks in Pediatric Brain Cancer

Author(s): Tobias Ehrenberger, Tenley C. Archer, Pablo Tamayo, Jill P. Mesirov, Scott L. Pomeroy, Ernest Fraenkel

Abstract:

Medulloblastoma (MB) is the most common pediatric brain cancer and has been studied extensively based on genomic, epigenomic (DNA methylation), and transcriptomic data. These studies found at least four disease subtypes with divergent molecular phenotypes and associated a subset of these subtypes with dysregulated signaling pathways (WNT and SHH) or specific altered gene expression signatures (Group3 and Group4). However, only few leads towards actionable targets have come out of these analyses. In this study, we are using integrative network modeling algorithms on a comprehensive set of functional ‘omics readouts, including proteomics, phosphoproteomics, and metabolomics, from a large cohort of primary MB tumors across subtypes to uncover signaling networks containing features that (1) will help us gain mechanistic insights into what drives the disease subtypes and (2) are of therapeutic interest.
Abstract:

Huntington’s Disease (HD) is a monogenic, progressive, autosomal dominant neurodegenerative disease caused by an elongated polyglutamine domain in the essential huntingtin protein. The mutated protein gains toxic new functions, but the mechanisms involved are not fully understood. Though there is no cure for HD, there are many compounds that have been shown to confer a protective effect in HD model systems. However, most of their mechanisms are unknown. We aim to identify the biological pathways targeted by these compounds in order to help design more effective methods for altering these pathways, and guide potential therapies.

We are performing “fingerprinting” assays to characterize the protective compounds into groups with similar transcriptional, epigenetic, and metabolic effects. Using representatives from each of the compound groups, we will perform detailed omic assays to gain a comprehensive understanding of the molecular changes involved in each compound treatment. This will allow us to infer each compound’s downstream effects. By applying integrated network analysis on the different omic data, we expect to find pathways altered by the compounds that could be responsible for their protective effects. Once validated, this approach could be used more generally for untargeted screens in other disease contexts.
Abstract Title: GATA1 and the Cohesin Complex Cooperate to Regulate Erythroid Gene Expression

Author(s): Gabriela Pregernig, Alireza Ghamari, Alan Cantor, Ernest Fraenkel

Abstract:

GATA1 is a transcription factor essential for erythropoiesis. It controls the expression of the majority of genes involved in red blood cell maturation, and mutations in its gene have been linked to several human hematopoietic diseases. Interestingly, GATA1 has been shown to serve both as a repressor and an activator, posing the question of how the direction of a gene's expression change is controlled.

Here, we present new evidence pointing to a functional relationship between GATA1 and the cohesin complex. We performed ChIP-Seq experiments against two members of the cohesin complex, SMC1 and SMC3, at 3 separate time points in a mouse erythroid cell line (G1E-ER4). We identified a large number of genomic regions that exhibit changes in cohesin occupancy throughout differentiation. To further investigate the top regions increasing in cohesin occupancy, we performed motif analysis, and found that the GATA motif was highly enriched. We also show that their neighborhoods are enriched for genes that are upregulated throughout erythroid development. We additionally collected GATA1 ChIP-Seq data, and observe that GATA1 co-occupies these same regions. Finally, preliminary immunoprecipitation results indicate that GATA1 physically interacts with several members of the cohesin complex. Together, these results suggest that GATA1 and the cohesin complex cooperate to regulate gene expression throughout erythroid development.
CEHS Lab Affiliation: Griffith

Poster Presented by: Victor Hernandez-Gordillo

Abstract Title: Toward the Development of an Organotypic Intestinal Model to Recapitulate Epithelial-stromal Interactions

Author(s): Victor Hernandez-Gordillo, Kelly Chen, Rebecca Carrier, John Hambor, Erick Young, Linda Griffith

Abstract:

Intestinal Organoids have gained interest as a tool to study intestinal biology and as a platform for drug screening because they resemble aspects of the intestinal complexity found in vivo. Organoids are cultured in ill-defined Matrigel-based hydrogels. Residual growth factors in Matrigel, lot-to-lot variability, the complexity of the media formulation, the variability in size and developmental stage of the organoids, and the limited access to the lumen, have prevented the widespread usage of intestinal organoids as a tool for drug screening. Furthermore, the stromal cells (myofibroblast and immune cells) that are important in disease models such as intestinal fibrosis are absent. Here, we report our strategy to engineer an in vitro model of the intestine that can recapitulate epithelial-stromal-immune interactions. The basic model will be constructed using epithelial cell derived from intestinal organoids, in combination with commercially available intestinal myofibroblast and immune cells using a layer-by-layer approach. In the advanced model, the stromal cells will be embedded in a well-defined semisynthetic matrix support with the epithelial cells seeding on top. The advanced model would allow investigating the influence of the microenvironment in the epithelial-stromal-immune interactions. Using conditioned medium that contains Wnt3a, R-spondin and Noggin we are able to create stable monolayers for up to 8 days. Upon differentiation, the trans-epithelial electrical resistance (TEER) is in the range of the native tissue (~300 ohms\(^2\text{cm}\)), suggesting the presence of tight junctions and a functional epithelial barrier. RT-PCR analysis also shows a decrease in LGR5 gene expression with a concomitant increase in Villin and Cyp3A expression. To complete the characterization the monolayers will be immunostained to identify the different cells types present and tested for the response to apical and basal stimuli (e.g. LPS) to evaluate the production of cytokines.
Abstract Title: Operational Strategies for Physiological Tissue Culture Medium of Microphysiological Systems using Primary Cells and Cell Lines

Author(s): Christian Maass, Matthew LaBarge, Matthew Dallas, Murat Cirit

Abstract:

Microphysiological systems (MPS) provide a more relevant physiological environment than current cell cultures. Current compositions consist of high glucose concentrations and regular complete media exchange (e.g. 48 h intervals) may prevent accumulation of waste products in the system. However, consumption rates of critical nutrients (e.g. glucose) or production rates of waste products (e.g. lactate) are not known for MPS systems and could shorten cell culture periods.

Thus, the aim of this study is to identify (1) glucose consumption and lactate production rates in a gut-MPS system and (2) an optimal dosing schedule using model-guided experimental design. Subsequently, this would allow to prolong the cell culture schedule and enable e.g. chronic toxicology studies as well as allow for an improved translation to in vivo.

Consumption and production rates were determined for a gut-MPS. Based on these rates, limiting nutrients and waste product concentrations were identified. An optimized experimental design was proposed using these rates to prolong the cell culture period in a more physiologically relevant environment and to allow for accumulation of relevant compounds.
CEHS Lab Affiliation: Griffith
Poster Presented by: Marianna Sofman

Abstract Title: The Development of a Facile Polymer Microbead-based Approach to Promoting Angiogenesis in Dense Epithelial Tissue

Author(s): Marianna Sofman, Alex Brown, Linda Griffith, Paula Hammond

Abstract:

3D in vitro tissue and organ cultures are of increasing interest as models for human disease and drug development. An enduring need is accomplishing vascularization of such tissues with perfusable capillary vessels. Insufficient vasculature within cultured tissue results in hypoxic conditions due to an oxygen transport limitation, hindering their survival, normal phenotypic outputs, and physiological function. Further, within the vascular niche of an epithelial system, the endothelial cells are surrounded by a vascular basement membrane, which provides mechanical stability, as well as various cytokines that support adjacent cell phenotypes. Epithelial systems such as liver, pancreas and other glands are particularly challenging, as these tissues are cell-dense with relatively little extracellular matrix; hence, successful vascularization approaches based on cell encapsulation or invasion into fibrin or other types of gels are difficult to adapt for these applications. Here, we harness concepts from the bead-based angiogenesis assays to design hydrogel microbeads with cell interaction properties tailored to promoting endothelial invasion into dense tissues. We use microfluidic-based polymer microbead synthesis, which combines single cell-based emergence with patterning techniques in order to actively vascularize dense cell tissue. Modulating the chemical and mechanical properties of the microbeads dictates cell adhesion, sprouting dynamics, and vascular density.
Abstract Title: Engineering Scaffold Vasculature Cues Using Projection Micro-Stereolithography

Author(s): Pierre Sphabmixay, Micha Sam Brickman Raredon, Donna Stolz, Alan Wells, Nicholas Fang, Linda Griffith

Abstract:

Introduction: While tissue engineering might reduce in the future the need for organ replacements and accelerate the development of drugs, it is greatly limited by its ability to generate thicker tissues due to the lack of vascularization\(^1\). A micro-machining technique featuring high resolution and high-throughput called Projection-micro-Stereolithography (PuSL) has been developed to improve vascularization cues for 3D in vitro perfused hepatic tissue. Fabrication of 3D structures using a bio-compatible and photo-polymerizable resin allows perfusion of dense cell mass with enhanced functionality and viability. Effective transport of nutrients through the tissue can be directed at sub-cellular resolution allowing to study the effects of mechanical cues and mass transport on angiogenesis. In that regard, the PuSL technology\(^2\) is able to generate cues to guide formation of a 3D-capillary network to efficiently perfuse 3D tissues. In this study, PuSL was applied to enhance distribution of perfusion by providing cues for angiogenesis from an initial source of endothelial cells.

Materials and Methods: Scaffolds were fabricated using the PuSL technology\(^3\). Simulations of the scaffolds were generated using COMSOL® 5.0 to predict fluid dynamics and oxygenation of the tissue using the model of a porous media and a Michaelis-menten kinetics for O2 consumption. The scaffolds were seeded with human cryopreserved hepatocytes (7.103 cells per construct) in co-culture with human dermal microvascular endothelial cells. The scaffolds were perfused in a bioreactor at a constant flow rate (1µL/s) over 5 days.

Results and Discussion: The PuSL apparatus was able to fabricate scaffolds with high resolution (down to 1.4 µm), high aspect ratio (up to 500 µm tall) and high throughput (1 min for single construct). The simulations predicted good oxygenation of the tissue (0.04 mol/m3 corresponding to hypoxia) with minimal shear stress distribution. Imaging of the tissues showed great viability and attachment of cells without need for coating or surface chemistry of the material, with endothelial cells associated with the scaffold cues.

Conclusions: The PuSL technology using a new photo-polymerizable resin achieved micro resolution while maintaining biocompatibility. The ability to construct hollow structures at this scale with high aspect ratio can be capitalized to readily perfuse tissues and direct flow in thick biological samples. In this study, the design principles were motivated by the maximization of the O2 while minimizing the shear stress distribution. Further study will focus on the effects of fluid dynamics and cell-cell interactions to promote angiogenesis.

Abstract Title: *A Microfluidic Mucosal Airway Model to Study Airway Mucosal Function and Pathogenesis*

Author(s): Chia-Chen Yu, Katharina Ribbeck, Jongyoon Han

Abstract:

Airway mucus hypersecretion or impaired clearance is one of the key pathophysiological features of airborne infection, allergy and severe respiratory diseases. As foreign particles enter the lungs, the airway becomes inflamed and excessive amount of mucus is generated in response. However, little is known about the role of mucus in regulating the passage of potentially harmful particles. In this work, we develop a novel in-vitro microfluidic system which closely models the biophysiological properties of the airway system. Our system accommodates co-current flow of aerosol and mucus and reproduces the key physiology of molecular and particle transport into a mucus barrier. A stable air-mucus interface with physiological clearance rate of mucus is achieved by optimizing device structural parameters. Evaporation-driven concentration is observed and limits our system to be used to model particle delivery to the nose and larger airways. With this platform, we hope to perform systematic permeability studies with diseased and normal mucus to develop a detailed understanding of the respiratory mucus permeability towards selected allergens. We envision the device to serve as a broad platform to identify key properties of mucus that may guide the way to improved airway disease diagnosis and treatment.
Abstract Title: A Fast, Portable, Fiber Optic Spectrofluorometer for Eddy Correlation Flux Measurement in the Aquatic Environment

Author(s): Harold Hemond, Irene Hu

Abstract:

The measurement of chemical fluxes between natural waters and their benthic sediments by most existing methods, such as benthic chambers and sediment core incubations, is slow, cumbersome, and often inaccurate. One promising new method for determining benthic fluxes is eddy correlation (EC), a minimally invasive, in situ technique based on high-speed velocity and concentration measurements. Widespread application of EC to a large range of chemicals of interest is currently limited, however, by the availability of rapid, high-resolution chemical sensors capable of precisely measuring concentrations at a point location and at sufficient speed (several Hz).

A prototype of an in-situ spectrofluorometry instrument has been created that is capable of high-frequency concentration measurements of naturally fluorescent substances. Designed with the EC application in mind, the system utilizes optical fibers to transmit excitation and emission light, enabling in situ measurements at high spatial resolution. Emitted fluorescence light is passed through a tunable monochromator before reaching a photomultiplier tube; photons are quantified by a custom miniaturized, low-power photon counting circuit board.

Used in an EC system, this instrument will enable flux measurements of substances such as naturally occurring fluorescent dissolved organic material (FDOM). This information in turn will allow the indirect measurement of numerous other benthic chemical fluxes, such as PCBs and PAHs, that we believe can be linked to those of FDOM by using tracer techniques. The use of a tunable monochromator not only allows flexibility in detection wavelength, but also enables full wavelength scans of the emission spectrum, making the spectrofluorometer a dual-function device capable of both characterizing the chemistry of the water and measuring fluorescence at selected wavelengths for EC and other applications.
CEHS Lab Affiliation: Kroll

Poster Presented by: Jonathan P. Franklin

Abstract Title: Measurements of I/SVOC from Mobile Sources Using Online Thermal Desorption EI-MS

Author(s): Jonathan P. Franklin, Eben S. Cross, Jesse H. Kroll

Abstract:

Increased exposure to ultra-fine particles (2.5 µm in diameter or smaller) is correlated with respiratory and cardiovascular disease. In urban settings, emissions from gasoline vehicles are significant sources of organic aerosol as well as precursors of secondary organic aerosol (SOA). Intermediate volatility/Semivolatile organic compounds, which are gas phase organic compounds with saturation vapor pressures between 10^{-3} and 10^{-6} µg/m³ are emitted from gasoline engines and are efficient precursors to SOA. However, a detailed understanding of such compounds in the atmosphere has been limited by the lack of fast and reliable measurements targeting I/SVOCs. In order to better understand the emissions of I/SVOCs from mobile sources, here we utilize a recently-developed online thermal desorption - electron impact mass spectrometric (TD-EIMS) technique to characterize the emissions from multiple gasoline vehicles. This instrument targets I/SVOCs by cryo-trapping samples and then thermally desorbing them into a high-resolution time-of-flight mass spectrometer. Measurements from 13 vehicles were characterized by the TD-EIMS and show the majority of IVOCs are emitted in the first minute of engine-on and contain a combination of aliphatic and aromatic organic compounds.
Abstract Title: **Integrated Gut/Liver Microphysiological System Elucidates Cytokine/Chemokine Inter-Tissue Crosstalk under Endotoxin-Induced Stress**

Author(s): **Kelly Wen Li Chen, Collin Edington, Emily Suter, Jeremy Velazquez, Rachel Dyer, Jason Velazquez, Michael Shockley, David Trumper, Rebecca Carrier, Murat Cirit, Linda G. Griffith, Douglas A. Lauffenburger**

Abstract:

Complex diseases often arise from network-level dysregulation as a result of perturbations across multiple tissues. The human gut is the largest immune organ in the body. Intestinal homeostasis is tightly regulated by the coordinated actions of a multitude of cell types, including epithelial cells, immune cells and the microbiome. Disruption of intestinal barrier function can trigger intestinal inflammation and potentiate extra-intestinal pathologies in downstream organs, such as the liver. However, a quantitative understanding of how these multicellular tissues communicate and contribute to overall (patho)physiology is limited.

To this end, we have developed and implemented a novel in vitro platform, together with the immune-competent human liver (hepatocytes and Kupffer cells) and intestinal models (enterocyte, goblet cells, and dendritic cells), to interrogate gut-liver interaction under normal and inflammatory contexts.

Our results demonstrated long-term (> 2 weeks) maintenance of intestinal (e.g., barrier integrity) and hepatic functions (e.g., albumin production) in baseline interaction. Interestingly, gene expression data comparing liver tissues in interaction versus isolation controls revealed significant modulation of bile acid metabolism pathway. Bi-directional gut-liver crosstalk potentiated feedback inhibition of hepatic Cyp7A1 expression, consistent with known physiology. Under inflammatory conditions, induced by endotoxin, non-linear modulation of cytokine responses was observed under interaction. In particular, CXCR3 chemokine ligands (e.g., CXCL9,10,11) production was significantly enhanced. Gene set enrichment analyses revealed significant upregulation of IFNα/β/γ signaling during inflammatory gut-liver crosstalk, with these pathways implicated in synergistic amplification of CXCR3 chemokine production. Furthermore, exacerbated inflammatory response in gut-liver interaction also detrimentally affected tissue-specific functions (e.g., reduction in liver lipid and drug metabolism). These findings illustrate how an integrated multi-tissue platform can generate insights potentially relevant for understanding complex pathophysiological processes such as inflammation-based organ crosstalk.
CEHS Lab Affiliation: Lauffenburger

Poster Presented by: Annelien J.M. Zweemer

Abstract Title: Apoptotic Cell Bodies Elicit Gas6-mediated Migration of AXL-expressing Tumor Cells

Author(s): Annelien J.M. Zweemer, Cory B. French, Joshua Mesfin, Simon Gordonov, Aaron S. Meyer, Douglas A. Lauffenburger

Abstract:

Metastases are a major cause of cancer mortality. AXL, a receptor tyrosine kinase (RTK) aberrantly expressed in many tumors, is a potent driver of metastatic cell motility and has been identified as broadly relevant in cancer drug resistance. Despite its frequent association with changes in cancer phenotypes, the precise events which lead to AXL activation are incompletely understood. In addition to its ligand Gas6, activation of AXL requires the lipid moiety phosphatidylserine (PS). PS is only available to mediate AXL activation when it is externalized on cell membranes, an event that occurs during certain physiologic processes such as apoptosis. We report here that exposure of cancer cells to PS-containing vesicles, including synthetic liposomes and apoptotic bodies, can contribute to enhanced migration of tumor cell lines via PS-Gas6-AXL signaling. Our findings suggest that anti-cancer treatments that induce fractional cell killing may enhance the motility of surviving cells in AXL-expressing tumors, which may explain the widespread role of AXL in limiting therapeutic efficacy.
Abstract Title: Genetic Validation and Prioritization of P. Falciparum tRNA Synthetases as Potential Antimalarial Drug Targets

Author(s): Sumanta Dey, Armiyaw Sebastian Nasamu, Sebastian Smick, Jacquin C. Niles

Abstract:

Plasmodium falciparum causes the most fatal form of malaria and is responsible for greater than 200 million infections annually and ~ 438,000 deaths. The dramatic rise in drug resistance poses a serious threat to global human health. Therefore, there is an immediate need for effective antimalarial drugs to control, prevent and eliminate malaria. tRNA synthetases (aaRS), which catalyze attachment of amino acids to their cognate transfer tRNAs, have emerged as a potentially important class of targets for antimalarial drug discovery. The P. falciparum genome encodes 37 aaRS, and these are integral to translation in the parasite’s cytosol, and its apicoplast and mitochondrial subcellular compartments. Structural differences between P. falciparum and human aaRS suggest that the parasite’s enzymes can be selectively targeted. However, while these enzymes are all predicted to be essential for parasite survival, and thus relevant as antimalarial drug targets, direct evidence supporting this assumption is lacking. Additionally, it is not always obvious that putative aaRS inhibitors specifically target these enzymes in parasites to exert their antimalarial activity. To address these questions, we are generating P. falciparum lines in which the expression of each aaRS gene can be conditionally regulated via a TetR-aptamer system we recently developed. We are using this resource to definitively establish the essentiality of the individual aaRS towards validating the subset that comprises the most robust biological targets for developing specific antimalarial drugs. We expect that this resource will rapidly facilitate identifying antimalarial compounds that target these enzymes, as well as evaluate the mechanism of antimalarial action of compounds developed as putatively specific aaRS inhibitors. Overall, we envision this resource will play an important role in prioritizing both drug targets and lead compounds towards discovering novel antimalarial drugs.
Calprotectin (CP) is a human protein that is released by neutrophils and epithelial cells at sites of infection as a part of the innate immune response. CP is a heterooligomer of S100A8 (α subunit, 10.8 kDa) and S100A9 (β subunit, 13.2 kDa). CP exerts antimicrobial activity by sequestering nutrient transition metals that are necessary for establishing infection. CP also binds Ca(II), which is plentiful in the extracellular space. Ca(II) binding causes self-association of two heterodimers to form an α₂β₂ tetramer that exhibits increased transition metal affinities, antimicrobial activity, and protease stability. Reactive oxygen species (ROS) also occur at sites of infection. In this work, we address how ROS affect the biophysical and functional properties of CP. Other research groups have performed mass spectrometry proteomics of human bodily fluids, and identified CP subunits bearing extra oxygen atoms, suggesting that oxidation of amino acid side chains by ROS has occurred (Martelli et al. (2016) J. Urol. 196, 911-918). Moreover, we have analyzed human nasal mucus by mass spectrometry, and identified oxidized CP subunits in some of the samples. We have found that treating recombinant CP with hydrogen peroxide oxidizes methionine side chains in each subunit to form methionine sulfoxide. We assayed the quaternary structural properties of oxidized CP, and discovered that the modified protein exhibited defective tetramerization and remained dimeric in the presence of Ca(II). Additionally, treating the CP heterotetramer with hydrogen peroxide caused dissociation of the tetramer. Protease degradation assays demonstrated that oxidized CP is more susceptible to proteolysis than the CP heterotetramer. Taken together, these observations indicate that post-translational oxidation of CP influences its oligomerization properties and protease stability. Despite its perturbed quaternary structural properties, oxidized CP exhibited the same antimicrobial activity as the unmodified protein. These findings shed light on a potential fate of CP in the extracellular space: ROS generated during infection and inflammation oxidize methionine residues of CP leading to accelerated proteolysis of the protein. It is possible that this putative degradation pathway is important for the homeostasis of extracellular CP, or in the case of dysregulated ROS, leads to premature degradation of CP.
Abstract:

Spontaneously occurring mutations accumulate in somatic cells throughout a person’s lifetime. Although most of these somatic mutations are harmless, occasionally a mutation affects a gene or a regulatory element and leads to a phenotypic consequence. Many ‘players’ maintain the equilibrium that determine mutation fixation in the genome. For example, genetic aberrations result from spontaneous mutations or from external carcinogens, and individual cells have the ability to deal with new mutations by fixing them with various DNA repair mechanisms or cell death. Breaking this equilibrium will cause mutation accumulation and will increase the chance to develop into cancer. Understanding the nature of human mutation accumulation is essential to improving healthcare through preventative medicine, detection of potential carcinogenic materials, understanding earlier stages of oncogenesis, ageing, etc.

We are developing a new DNA sequencing approach, ‘lineage sequencing’, which carries out independent genome sequencing of known lineages in a clonal population and promises improved sensitivity and specificity of genomic analyses. Lineage sequencing works in a controlled genetic background and uses expectations about inheritance states, such that every lineage of the pedigree is processed, enabling reconstruction of the entire history of mutations in the clonal population of cells. The dataset has single generation resolution of mutations occurring during the growth of the initial population, and is extraordinarily accurate, far superior to standard sequencing approaches due to error correction enabled by the unique structure of lineage sequencing data.

We will use this method for measuring the effect of exogenic mutagenic stress, by combining comparable measurements of the effect of chemical perturbations on mutation accumulation, and follow the mutagenesis process over 3 generations. As new generation of genotoxicity test, by performing a measureable exogenic exposure in a specific control manner during lineage progression in known ‘time frames’.
Abstract Title: Impact of Unconventional Oil and Gas Drilling on Soil and Water Quality in Pennsylvania

Author(s): Neha Mehta, Charles Harvey, Benjamin D. Kocar

Abstract:

Wastewater produced in hydraulic fracturing operations contains a complex milieu of anthropogenic and natural chemical constituents, including naturally occurring toxic inorganic elements such as strontium, barium and naturally occurring radioactive materials such as radium. Discharge of contaminated fracturing wastewater to surficial soils is a frequent occurrence; hundreds of spills have been recorded, ranging from several to millions of gallons of wastewater, causing deleterious effects on soil and water quality. In this study, we present data on soil and stream water quality in vicinity of active hydraulic fracturing operations located in Tioga County, Pennsylvania. In all samples, radium isotopes are measured using γ spectroscopy, and samples are evaluated for the presence of co-contaminants, including inorganic Ba, Sr, As, U, Fe and Pb (using ICP-MS), which, depending on geochemical conditions, undergo appreciable sorption to soils and sediments and potentially bio-accumulate across food chain. We also present an approach to distinguish hydrofracturing contamination at a spill site by comparing radium isotopic signature in soils and in shale cores collected from Marcellus shale formation underlying Pennsylvania. Our results suggest that chemical and radiological signatures of hydraulic fracturing are observed in soils and natural waters adjacent to areas where active drilling has occurred, warranting further efforts to decipher the areal extent of contamination throughout regions where unconventional gas extraction has occurred.
Abstract Title: Quantifying Population Exposure to Fine Particulate Air Pollution Using an AOD-LUR Combined Model, and Mobile-Device Based Human Mobility Patterns

Author(s): Marguerite Nyhan, Itai Kloog, Petros Koutrakis, Carlo Ratti

Abstract:

Air pollution is recognized by the World Health Organization as the world's leading environmental and human health threat. In previous environmental epidemiological studies linking air pollution and human health end-points, exposure estimates at the population level have not considered individuals’ spatially- and temporally varying mobility patterns. In the first study of its kind, we use measured mobility patterns of almost half a million people resident and working in the Greater Boston Area, to evaluate short- and long-term exposures to PM2.5. Mobile and wireless devices yield information about where and when people are located, thus extensive cellular network data were used to characterize daily mobility patterns of 447,435 people. Individual’s short-term and long-term exposures to PM were quantified. The exposures assigned, herein categorized as ‘Active Exposure’, were based on the home location, work location and the commuting pattern identified. These exposures were compared to the ‘Home Exposure’, which assumed individuals remained at their residential location for the entire study duration; consistent with previous major environmental epidemiological cohort studies. Spatial and temporal variations in PM2.5 concentration levels were predicted using a satellite Aerosol Optical Depth and Land Use Regression- combined model. In comparing the daily ‘Active Exposure’ to the ‘Home Exposure’ computed for each of the 447,435 individuals for one year, statistically significant (p<0.05) differences were determined for 22% of the population (98,436 people). In examining the long-term PM2.5 exposures computed for the population during the ‘Active’ and ‘Home’ scenarios, differences ranged from 0 to 3.4 µg/m³ annually, while the range of differences was smaller during the summer (0 to 2.7 µg/m³) and larger during the winter (0 to 3.4 µg/m³). Cellular network data may be used to improve the accuracy of short- and long-term air pollution exposure estimates of large populations. It presents a significant step forward in exposure science and warrants consideration in prospective environmental epidemiological studies linking air pollution and human health end-points.
CEHS Lab Affiliation: Pentelute
Poster Presented by: Ethan Evans

Abstract Title: Toward the Rational Design of Reactive Miniproteins
Author(s): Ethan Evans

Abstract:
The ability to discover and design reactive biomolecules is a fundamental test of the scientific community’s understanding of the underlying system physics and/or statistical distributions. This talk describes the beginning steps to understand and then design a reactive miniprotein capable of self-labeling via perfluoroaryl-based SNAr chemistry. A single progenitor sequence is analyzed and characterized, first via alanine scan mutagenesis and structure prediction. The design process is then initiated to uncover more reactive miniprotein sequences using a mixture of computational and experimental tools.
Abstract:
The physicochemical properties of mucus barriers are intricately related to physiology, but non-invasive strategies to detect diseases using mucus as a diagnostic are largely underdeveloped. The mechanical properties of healthy cervical mucus, for instance, are dependent on reproductive physiology, while pathological onset of mucus barrier dysfunction can lead to a number of devastating pulmonary, gastrointestinal and urogenital conditions. Here, we demonstrate this diagnostic potential by interrogating the physicochemical properties of cervical mucus from ovulating, low risk pregnant, and high risk pregnant patients. At the micron length scale where steric exclusion is believed to be the dominant barrier mechanism, we show using single particle tracking (SPT) that the motion of negatively charged probes is significantly restricted in cervical mucus from pregnant patients as compared to non-pregnant patients. Although risk of preterm birth cannot be distinguished at this length scale, we demonstrate using a peptide reporter system that cervical mucus from low risk pregnancy patients is more effective at excluding negatively charged probes and at limiting the diffusion of positively charged ones than mucus from high risk pregnancy patients, suggesting that biochemical changes may be important contributors to the altered function of the mucus barrier during preterm birth. Furthermore, we demonstrate for the first time that these changes in permeability correlate quantitatively with progesterone levels. We expect that these findings have important implications for understanding disease progression on all mucosal epithelia, including those found in the lungs and gut, and enable the more rapid development of diagnostic tools that utilize mucus as a source of biophysical indicators for health and disease.
Abstract Title: **Swimming Bacteria promote Dispersal of Non-motile Staphylococcal Species**

**Author(s):** Tahoura Samad, Nicole Billings, Alona Birjiniuk, Thomas Crouzier, Patrick S. Doyle, Katharina Ribbeck

**Abstract:**

Swimming motility is considered a beneficial trait among bacterial species as it enables movement across fluid environments and augments invasion of tissues within the host. However, non-swimming bacteria also flourish in fluid habitats, but how they effectively spread and colonize distant ecological niches remains unclear. We show that non-motile staphylococci can gain motility by hitchhiking on swimming bacteria, leading to extended and directed motion with increased velocity. This interaction was observed between S. aureus and P. aeruginosa, S. epidermidis and P. aeruginosa, as well as S. aureus and E. coli, suggesting hitchhiking as a general translocation mechanism for non-motile staphylococcal species. By leveraging the motility of swimming bacteria, it was observed that staphylococci can colonize new niches that are less available in the absence of swimming carriers. This work highlights the importance of considering interactions within polymicrobial communities, in which bacteria can utilize each other as resources.
Abstract Title: Necroptosis and Inflammation Mediates Alkylation-induced Retinal Degeneration in Mouse

Author(s): Mariacarmela Allocca, Joshua J. Corrigan, Aprotim Mazumder, Leona D. Samson

Abstract:

Alkylating agents represent one class of commonly-utilized chemotherapeutic agents that generate numerous types of alkylated DNA base lesions. Although the base excision repair (BER) pathway can repair DNA alkylation damage, under certain conditions, the initiation of BER produces toxic repair intermediates that damage healthy tissues. We have shown that the alkyladenine DNA glycosylase Aag (a.k.a. Mpg), an enzyme that initiates BER, mediates alkylation-induced retinal degeneration (RD). RD is wholly prevented by Aag deficiency. The purpose of this work was to study the involvement of necroptosis and inflammation in alkylation-induced RD.

RD was induced by intraperitoneal injections of the alkylating agent, methyl-methanesulfonate (MMS). Morphological and biochemical assessment of degeneration, necroptosis and inflammation was performed by electron microscopy, histology, immunofluorescence and gene expression analyses.

MMS-injected male mice showed a reduced number of photoreceptor (PR) nuclei, active gliosis, macrophages infiltration into the outer retina and vacuolated retinal pigment epithelial cells (RPE) as early as 3 days post-treatment. Ultra-structural analysis of retinas revealed that a fraction of dying PR exhibited necrotic morphology. The expression of RIP1 and RIP3, key regulators of necroptosis, was elevated in the neural retina but not RPE cells. Moreover, poly(ADP-ribose) polymerase (PARP) activity was localized to PR cells and associated with release of high-mobility group-1 (HMGB1) from PR nuclei. High levels of the pro-inflammatory cytokine TNFα and the chemokine CCL2 as well as increased levels of anti-inflammatory cytokines such, as IL-10, were also observed in the neural retina. Moreover, deficiency of IL-10 resulted in more severe RD while deficiency of RIP3 resulted in partial protection. Female mice were partially protected from MMS-induced RD and showed reduced evidence of necroptosis and inflammation when compared to males. Aag/- mice did not show any sign of necroptosis or inflammation.

Our findings provide in vivo evidence that alkylating agents induce sex-dependent RD mainly by induction of necroptosis in PR cells and activation of the inflammatory response. Given the extensive use of alkylating agents as chemotherapeutics and the wide range of Aag activity in the human population, understanding the molecular mechanisms underlying alkylation-induced PR cell death may be crucial to prevent potential side-effects.
Abstract Title: NRF2 and Glutathione are Key Resistance Mediators to Temozolomide in Glioma and Melanoma Cells

Author(s): Clarissa R. Rocha, Gustavo S. Kajitani, Annabel Quinet, Rodrigo S. Fortunato, Carlos F. Menck

Abstract:

Cancer is a leading cause of death worldwide, and while great advances have been made particularly in chemotherapy, many types of cancer still present a dismal prognosis. In the case of glioma, temozolomide (TMZ) is the main option for treatment, but it has limited success due to drug resistance. While this resistance is usually associated to DNA repair mechanisms, in this work we demonstrate that oxidative stress plays an important role. We showed that upon TMZ treatment there is an induction of the nuclear factor erythroid 2-related factor 2 (NRF2), which is the main antioxidant transcription factor regulator in human cells. This is accompanied by an enhancement of glutathione (GSH) concentration in the tumor cells. The effectiveness of this pathway was proven by silencing NFR2, which greatly enhanced cell death upon TMZ treatment both in vitro and in vivo. Also, higher DNA damage and induced cell death was observed by combining BSO - a GSH inhibitor - with TMZ. Similar effects were also observed using in vitro and in vivo models of melanoma, thus possibly indicating that GSH has a decisive role in TMZ resistance in a wider range of tumors. Thus, a combined regimen of BSO and TMZ configures an interesting therapeutic alternative for fighting both glioma and melanoma.
Abstract Title: Does More Complex Chemistry Always Lead to a Better Result? Exploring Air Quality and Human Health Uncertainties Resulting from the Choice of Chemical Mechanism

Author(s): Benjamin Brown-Steiner, Noelle Selin, Ron Prinn, Erwan Monier, Simone Tilmes, Louisa Emmons

Abstract:

Continuing advancements in computational capabilities has enabled the atmospheric chemistry and climate research community to incorporate more detailed and complex components and parameterizations and to increase the resolution and length of their simulations. This has enabled more detailed and more advanced studies of the impact of air quality on human health, but the computational cost of these simulations is high and can limit the length of a simulation or the number of simulation members in an ensemble. Here we examine the potential benefits and uncertainties provided by three chemical mechanisms of different complexities (full tropospheric, reduced hydrocarbon, and a superfast mechanism) within the CESM CAM-Chem framework and characterize the research questions that can most benefit from longer simulations of simpler mechanisms. We demonstrate that uncertainties associated with the utilization of different chemical mechanisms is less important in the characterization of ozone distributions and variability than the choice of meteorological dataset, and that a calibration process can sufficiently emulate the behavior of the complex mechanism. We offer recommendations and suggestions for the length of time, the averaging window, and the size of the region in which this process is sufficient and propose to extend this analysis to other model uncertainties.
CEHS Lab Affiliation: Shoulders

Poster Presented by: Christopher L. Moore

Abstract Title: Multidimensional Chemical Control of CRISPR-Cas9

Author(s): Christopher L. Moore, Basudeb Maji, Amit Choudhary, Matthew Shoulders

Abstract:

Facile Cas9-derived technologies have revolutionized the gene targeting landscape for functions ranging from DNA editing to transcriptional regulation. However, a critical need still exists for robust methods to regulate the activities of these endonuclease-based tools in living systems. In the context of gene editing, small molecule-based tools could be utilized to regulate the levels and/or the activity of endonucleases to enhance specificity and provide temporal control of gene editing. For gene regulation, complete temporal control of transcription induction is essential for the study cell signaling, stress response pathways and other complex biological systems. Furthermore, dosable control of transcriptional activity is absolutely essential to ensure that physiologically relevant levels of gene expression are achieved. In order to address these technical needs, we designed a broadly applicable method for controlling applied activities of RNA-guided nuclease biology by integrating destabilized domain technology with the CRISPR/Cas9 system. We find that incorporation of destabilized domain and CRISPR/Cas9 transcriptional perturbation technologies provides users with a convenient method for controlling many aspects, including time and extent of endogenous gene upregulation, through addition or removal of a small molecule. For gene editing, we find that destabilized domain Cas9 confers provides users with exquisite control parameters and reduces off-target effects. Taken together, these small molecule-regulated CRISPR/Cas9 tools allow us to carry out genetic forward screens to determine how particular genes are involved in complex biological responses to environmental stresses.
Abstract Title: Host Protein Homeostasis Network-Mediated Modulation of Influenza Evolution

Author(s): Angela M. Phillips, Luna O. Gonzalez, Anna I. Ponomarenko, Emmanuel E. Nekongo, Vincent Butty, Stuart S. Levine, Leonid A. Mirny, Matthew D. Shoulders

Abstract:

Broad and rapid exploration of the mutational landscape by RNA viruses like influenza engenders high adaptability to environmental challenges. Understanding, predicting, and constraining RNA virus evolution requires a comprehensive picture of the molecular factors that define the accessible mutational landscape. Both the biophysical properties of viral protein variants and the ability of viruses as obligate parasites to hijack host protein folding mechanisms are expected to be important factors in this regard. Here, we test the hypothesis that the composition and activities of the host proteostasis network, comprised of chaperones and quality control factors, can constrain and enable influenza evolution by both modulating overall selection pressure and by altering the fitness of specific viral protein variants. Chemical genetic and small molecule methods allow us to create divergent host cell proteostasis environments via either the stress-independent activation of heat shock factor 1 (HSF1) or the inhibition of Hsp90 chaperones. Extensive propagation of influenza virus populations in these modified host environments reveals that host protein folding mechanisms influence both the selection on the influenza genome and the accessibility of specific mutational trajectories for select influenza proteins.
CEHS Lab Affiliation: So

Poster Presented by: Dushan N. Wadduwage

Abstract Title: Gamma-H2AX foci Quantification Using High-throughput 3D Image Cytometry

Author(s): Dushan N. Wadduwage, Marcus Parrish, Bevin P. Engelward, Paul Matsudaira, Peter T.C. So

Abstract:

Ionising radiations cause various types of DNA damages including double strand breaks (DSB) that are often recognized by DNA repair protein ATM which forms gamma-H2AX foci. Quantification of gamma-H2AX foci is usually done using flow cytometry or confocal imaging followed by spot counting. Flow cytometry lacks counting accuracy as the foci cannot be resolved, and confocal imaging lacks statistical accuracy as the number of cells that can be analysed is limited. Here we present a high-throughput method using an in-house developed 3D image cytometer based on structured light illumination, remote depth scanning, and an automated image processing pipeline. The instrument can image about 800 cells per second in 3D at diffraction-limited resolution. The image processing pipeline can count over 100 foci per nucleus implying better quantification. Together, the approach offers increased dynamic range for DNA damage quantification and increased statistical accuracy by enabling analysis of hundreds of thousands of cells per experiment.
Abstract:

Carbon nanomaterials such as CNTs and graphene have attracted immense interest because of their outstanding electrical and mechanical properties. Despite the great potential of these materials towards applications in sensing, catalysis, and future electronic devices, the pristine material’s poor solubility and the lack of specificity have limited their use. Covalent functionalization allows the surface of CNTs and graphene to be modified with different chemical groups. These approaches increase the solubility as well as offering the capability to tailor the properties of these materials for specific function. Here we present a novel method for the covalent functionalization of CNTs and graphite using aryl iodonium salts.
Abstract Title: Reprogramming the SNO-Proteome in the brain of the Shank3-KO model of Autism Spectrum Disorder

Author(s): Haitham Amal, Boaz Barak, Vadiraja Bhat, John S. Wishnok, Guoping Feng, Steven R. Tannenbaum

Abstract:

It is well established that Nitric Oxide (NO), and especially S-nitrosylation of key neuronal proteins play a key role in several neuropathologies. Autism Spectrum Disorders (ASDs) are neurodevelopmental disorders based on three behavioral characteristics: impaired social interaction, lack in communication and repetitive or restricted behavior. One high confidence ASD mutated gene is Shank3, which codes for a postsynaptic scaffolding protein and plays a critical role in neuronal development. Synaptic transmission is one of the major malfunctioning processes in ASD.

Recently, several groups have developed high throughput mass spectrometric approaches for analysis of the SNO-proteome in different tissues, including the brain that has revealed hundreds of proteins associated with a pathological state. This has allowed analysis of the role of SNO on a systems level. To test our hypothesis that NO is involved in ASD, and to unravel its effect on synaptic transmission and signaling pathways, we used a Shank3-KO mouse model.

Using SNOTRAP (SNO trapping by TriAryl Phosphine), we have tested two mouse age groups (Adults and 6 weeks) in two brain regions, cortex and striatum.

In the adults, we found a large increase of SNO-proteins in the KO model compared to the WT in both cortex and striatum, while in 6 weeks mice, we found a large increase in the cortex with a smaller increase in the striatum.

Gene Ontology analysis showed that the SNO-proteome in the KO-Adults-Cortex identified SNO-proteins that are critical and important to neuron projection, post-synaptic density, synaptic vesicle fusion, nervous system development, synapse, myelin sheath, glutamate biosynthesis and other neuronal related processes. None of the above appeared in the WT-Adults-Cortex. Specific proteins were identified in the KO-6weeks-Cortex that are related to the glutamatergic synapse, neuron projection, myelin sheath, regulation of signal transduction, and nervous system development, none of which appeared in the WT-6 weeks-Cortex.

A key protein that was S-nitrosylated in the KO group in both adults and 6 weeks ages was Syntaxin1A which functions in the synaptic vesicle fusion process. S-nitrosation of Syntaxin1A facilitates its engagement with the membrane fusion machinery and thus enhances excessive vesicle fusion. This mechanism would shed light on the imbalanced excitatory and inhibitory post-synaptic potential and synaptic transmission in ASD. Another protein that S-nitrosylated in both KO ages is Serine/threonine-protein phosphatase 2B which is the catalytic subunit of Calcineurin. The PKA signaling cascade is negatively regulated by Calcineurin.

S-nitrosation of this phosphatase may inhibit its activity and lead to up-regulation of PKA which is correlated with different neurological disorders. In conclusion, our findings could lead to a comprehensive analysis of
Abstract Title: **Interleukin-22 Drives Nitric Oxide-Dependent DNA Damage and Dysplasia in a Murine Model of Colitis-associated Cancer**

Author(s): Guanyu Gong, Chuan Wang, Alexander Sheh, Sureshkumar Muthupalani, Erin M. Bryant, Dyan A. Puglisi, Hilda Holcombe, Evan A. Conaway, Nicola A. Parry, Vasudevan Bakthavatchalu, Sarah P. Short, Christopher S. Williams, Gerald N. Wogan, Steven R. Tannenbaum, James G. Fox, Bruce H. Horwitz

Abstract:

The risk of colon cancer is increased in patients with inflammatory bowel disease. Inflammation-induced DNA damage are suspected to be an important link between inflammation and cancer, although the exact pathways responsible for these changes are incompletely defined. 129RAG2-deficient mice infected with Helicobacter hepaticus (Hh) develop colitis that progresses to lower bowel cancer. This process depends on nitric oxide (NO), a molecule with known mutagenic potential. We have previously shown that production of NO and HOCl leads to mutagenic changes to nucleotides during Hh-infection, however, whether these changes result in DNA damage in cells, the primary sources of these free radical molecules, and whether the DNA damage depends on NO/HOCl/both, have not been determined. Here, we demonstrate that Hh infection of RAG2-deficient mice rapidly (2 weeks and 10 weeks post infection) induces expression of iNOS and the development of DNA double-stranded breaks (DSBs) specifically in proliferating crypt epithelial cells. Generation of DSBs depended on iNOS activity; further, induction of iNOS, the generation of DSBs, and the subsequent development of dysplasia were inhibited by depletion of the Hh-induced cytokine IL-22 (both 2 weeks and 10 weeks). In contrast, IL-22 depletion results in minimal changes in myeloperoxidase-positive neutrophils and depletion of neutrophils did not prevent DNA damage in the epithelial cells. These results demonstrate a strong association between Hh-induced DNA damage and the development of dysplasia, and further suggest that IL-22 dependent induction of iNOS within crypt epithelial cells rather than macrophages is a driving force in this process.
Abstract:

Metabolomics, the “global” study of metabolite changes in a biological system, has drawn a significant amount of interest over the last few years. It can be said to be an amalgam of traditional areas such as metabolite analysis, bioanalytical development and chemometrics. Most work to date has been focused on plant, tissue, as well as biofluid samples. However, the diverse potential of metabolomics in many fields, including cell engineering, has made it a universal tool for industrial, medical and research purposes. So here, we look at the cell-culture applications of metabolomics and our results confidently support that metabolomics is a crucial tool for the investigation of cell engineering and drug development.

Among all the biological systems in our body, the human brain is particularly sensitive to toxic insults during development. Stem cell-derived model of the human central nervous system (CNS model) has dramatic potential to improve our understanding of brain. Considering the interactions between multiple human organs, we use this CNS model in conjunction with a human-on-a-chip system, which includes pancreas, liver, gut, lung, heart, brain and endometrium models (7-way platform), for biomarker analysis and metabolomics study.

N-Acetylaspartic acid (NAA) was selected as a neuronal marker to track cells as they develop. We have established a sensitive and robust mass spectrometry method for NAA quantification in brain samples with good linearity ($R^2 = 0.9997$). The limit of detection ($S/N = 3$) of NAA was only 2.3 nM and the limit of quantification ($S/N = 10$) was found to be 7.5 nM. Subsequently, typical brain samples from Lag phase (Day 10), Log phase (Day 18) and Stationary phase (Day 28) were selected for time-dependent metabolomic analysis. Results show that the sphingolipid, glycerophospholipid, beta-Alanine and glutathione metabolism were perturbed during cell culture.

In the end, toxicity test was proceeded in order to see how the interactive, multi-organ system responds to toxic insults. The human-on-a-chip system samples were dosed with 30 Cmax Tolcapone (TCP). We also conducted metabolomic analysis between the brain samples with TCP dosing and the samples without TCP. 9 specific metabolic pathways were significantly perturbed from blank controls. Among them, the phenylalanine metabolism and folate metabolism are highly relevant for the efficacy of drug; while the perturbations of glycerophospholipid metabolism, citrate cycle, glycolysis and gluconeogenesis, and metabolism of some neurotransmitters are related to the toxicity of the dose. Suggesting that both pharmacological and toxic effects of TCP are investigated at the same time in this powerful metabolomics study.
CEHS Lab Affiliation: Walker

Poster Presented by: Nimrat Chatterjee

Abstract Title: A Stapled Pol κ Peptide Binds Rev1 And Enhances Cellular Toxicity

Author(s): Nimrat Chatterjee, Sanjay D’Souza, Mohammad Shabab, Gerard Heliniski, Gregory L. Verdine, Graham C. Walker

Abstract:

Cancer drug resistance is a persistent clinical problem, one that limits successful treatment in patients. Previously we showed that suppression of Rev1 translesion DNA polymerase inhibits both cisplatin and cyclophosphamide induced mutagenesis thereby causing increased cell death. Likewise, presence of Rev1 was shown to promote the development of acquired drug resistance and its suppression increased survival in mice. It is not known whether by targeting Rev1 at specific regions, which are important for its translesion function, would also promote cell toxicity. Here, we demonstrate that a staple Pol κ peptide, with sequences derived from the RIR (Rev1 interacting region), binds strongly to Rev1 C-terminus and is capable of enhancing cisplatin-induced cell toxicity in different cell types. Further, this staple peptide also sensitizes cells to different types of carcinogens. This study shows that by targeting specific interaction regions of Rev1, translesion synthesis can be suppressed, which potentially opens up new avenues of preventing tumor chemoresistance.
Abstract Title: Lethality Caused by MalE-LacZ Hybrid Protein Shares Attributes with Oxidative Component of Cell Death Caused by Bactericidal Antibiotics

Author(s): Charley C. Gruber, Noriko Takahashi, Chittampalli Yashaswini, Jason H. Yang, Xiaobo Liu, Dana Braff, Sakkarin Bhubhanil, Silvana Andreescu, James J. Collins, Graham C. Walker

Abstract:

It has been reported that different lethal stressors such as bactericidal antibiotics, bacteriophages, and the T6SS lead to the production of reactive oxygen (ROS) species which contribute to their lethal effects. MalE-LacZ was a fusion protein created to study protein secretion and was used to identify the signal sequence and components of the general secretory pathway. Expression of this protein is lethal to cells due to jamming the secretion apparatus, but the exact mechanism of cell death involved is unknown. Here we report that induction of this protein leads to the production of ROS which contribute to cell death in a DNA repair dependent manner.
Abstract Title: mTOR is a Novel Synthetic Lethal Partner of Rev3 that can Enhance Cisplatin Sensitivity in Lymphoma Cells

Author(s): Kinrin Yamanaka, Michael Hemann, Graham Walker

Abstract:

Translesion DNA synthesis (TLS) is a mechanism that helps cells tolerate unrepaired DNA lesions through replication of damaged DNA and is carried out by TLS polymerases. A growing body of evidence shows that suppression of TLS polymerases not only sensitizes tumor cells to drugs, but also reduces acquisition of drug-induced mutations implicated in tumor resistance. Therefore, inhibition of TLS polymerases is promising new approach to cancer therapy. Rev3 is a catalytic subunit of TLS polymerase zeta that plays a major role in mutagenic TLS. Multiple genes are often mutated in cancers. Identifying genetic background of cancer cells in which Rev3 inhibition is most effective is thus crucial. Here we carried out shRNA library screen in search for gene(s) that exhibit synthetic lethal interactions with Rev3 in response to cisplatin in lymphoma cells. mTOR was identified to be a novel synthetic lethal partner of Rev3 that could enhance cisplatin sensitivity in lymphoma cells. Additionally, we demonstrate that concomitant suppression of Rev3 and mTOR increased cisplatin-induced cell death through necrosis, and an increased DNA damage in the form of double-strand break was observed. This is the first report to demonstrate the cooperative functions of Rev3 and mTOR to sensitize cancer cells to cisplatin.
Abstract Title: Phosphotyrosine Signaling in Alzheimer’s Disease

Author(s): Nader Morshed, Lauren J. Perez, Alexi Nott, Li-Huei Tsai, Forest M. White

Abstract:

Alzheimer's disease is the leading cause of dementia worldwide, with no effective treatments for preventing disease. Despite recent progress in deciphering the genetics of this disease, there remains a dearth of knowledge on how these factors fit into the larger signaling networks in the brain. To address this gap, we have quantified the changes in protein phosphorylation induced in a mouse model of disease. This uncovered a set of phosphorylation sites changing in the microglial cells thought to respond to toxic events in the brain.