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Poster Presentations

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G-quadruplex in parp1-mediated DNA damage response

Andrea D Edwards, Alicia K Byrd, John C Marecki, and **Kevin D Raney**
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Each cell in the human body can receive tens of thousands of DNA lesions resulting in genomic instability and disease. The DNA damage response is a fundamental cellular system that protects cells from endogenous and exogenous insults to DNA. Though repair pathways are known, the mechanism of sensing, signaling, and repair are not completely defined. We have gathered evidence for a novel signaling mechanism functioning through G-Quadruplex (G4DNA) sequences. G4DNA is found in key regulatory regions of the cell such as promoters of proto-oncogenes and telomeres. Using a monoclonal antibody to folded G4DNA, our data suggest that the presence of G4DNA quadruplexes decrease in the nucleus with a concomitant increase in the cytoplasm during oxidative stress. Base excision repair (BER) is known to function on oxidized DNA bases such as 8-oxoG. Therefore, in order to determine why oxidative stress leads to the reduction of G4DNA quadruplexes in the nucleus, we are focusing on proteins involved in BER. Poly (ADP-ribose) polymerase 1 (PARP-1) is a DNA damage response protein that functions in BER and has been shown to bind G4DNA. The enzymatic activity of PARP-1, termed PARylation, is necessary for proper function of BER. Our preliminary data suggest that PARP-1enzymatic activity is stimulated by G4DNA only when the G4 structure contains one or more single-stranded DNA loop regions of >1 nucleotide. It has been shown by others that damaged guanines within G4DNA are extruded out of the quadruplex structure into long single-stranded DNA loops. We hypothesize that PARP-1 binds G4DNA and is enzymatically activated by the presence of a ssDNA loop extrusion that occurs when G4DNA is damaged during oxidative stress. We will measure binding between PARP-1 and various G4DNA substrates containing oxidized guanines and be varying single-stranded DNA loop lengths in order to test this hypothesis.

Biography

Andrea Edwards is a current graduate student at the University of Arkansas for Medical Sciences. She works under the advisement of Dr Kevin Raney in the Department of Biochemistry and Molecular Biology. Her current research is focused on providing further insight into the DNA damage response. Her area of interest includes G-Quadruplex sequences. These sequences occur in important regions of the genome and are also susceptible to oxidative damage. Her research goal is to determine the repair pathway involving these sequences. Mutation of these promoters within several proto-oncogenes has been associated with numerous cancers. Her goal is to provide information that could be used to develop better therapeutics targeted to cancer.

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Hepatitis C virus non-structural protein 3 (HCV NS3) resolves G4RNA structures

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Hepatitis C virus (HCV) is the major cause of chronic liver disease and hepatocellular carcinoma. It is currently estimated that over 71 million people in the world are infected with HCV virus. The HCV genome encodes for a polyprotein that is cleaved into 7 non-structural and 3 structural proteins. One of the non-structural proteins, non-structural protein 3 (NS3), has both protease and helicase domains and is the key protein in regulating HCV RNA replication. NS3 has been one of the major targets in the treatment of HCV infection. The current HCV drugs are extremely expensive and patients may develop resistance. Therefore, it is important to develop a cost-effective anti-viral compound that targets conserved regions within the HCV genome and prevent replication of various HCV genotypes and subtypes. Recent works have shown the presence of conserved guanine-rich consensus sequences within the HCV genome. These guanine sequences can form G-quadruplex (G4) secondary structures through Hoogsteen hydrogen bonding. It has been reported that both replication and translation of the HCV genome could be inhibited by the formation of G4 structures. However, it is still unknown how HCV G4RNA structures are regulated. The aim of this project is to investigate whether and how NS3 helicase resolves HCV G4RNA structures. Understanding the mechanism by which NS3 resolves HCV G4RNA might allow us to find processes and factors that could be targeted to prevent HCV replication in a host cell.

Biography

Binyam Belachew is a second year Ph.D. student in Kevin Raney's laboratory. He graduated from Wingate University (NC) in 2014 with a Bachelor of Science in Biology and a minor in Chemistry. As an undergraduate student, he conducted both Chemistry and Biology research with Wingate University professors; He also served as a laboratory assistant. Following his undergraduate program, Binyam worked as a laboratory analyst II for Charlotte Water Company in Charlotte, North Carolina before moving to Little Rock, Arkansas for his doctoral program at the University of Arkansas for Medical Sciences.

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The potential protective role of urokinase plasminogen activator against oxidative induced DNA damage in periodontal ligament tissue

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There is a high concentration of urokinase plasminogen activator (uPA) in gingival cervical fluid and saliva. However, still not much is known about the physiological role and function of this serine protease in oral tissue. The aim of the present study was to investigate the effect of urokinase in DNA damage and repair process outcome after exposure to oxidative stress in fibroblasts derived from periodontal ligament (PDL) and to propose a new protective role for uPA in an oral cavity. To this aim, PDL fibroblasts were isolated from human wisdom teeth of healthy donors and were pre-incubated with urokinase or amiloride, a urokinase inhibitor, followed by exposure to the different concentrations of H2O2. After peroxide treatment, cells were re-incubated with urokinase. Cell viability and apoptosis were assessed by MTT and Anexin V/PI assay, respectively. The level of γH2AX expression was studied as a sensitive marker of DNA damage using immune staining and flow cytometry. Alkaline comet assay was performed to detect DNA damage single and double-strand breaks. Our results showed that pre-incubation of the peroxide-treated cells with urokinase significantly increased cell viability and decreased cell apoptosis. Furthermore, there was a significant decreased in the expression of γH2AX after peroxide treatment in cells incubated with urokinase. Oxidative stress-induced DNA damage breaks were reduced in urokinase-treated groups as measured by comet assay. However, inhibition of urokinase with amiloride, in turn, resulted in higher level of DNA damage breaks and increased apoptotic cells after exposure to peroxide. The present study suggested a new protective role for urokinase plasminogen activator against oxidative-induced DNA damages in oral tissue. As the oral cavity is constantly exposed to the oxidative agents, decreased in urokinase expression in saliva might be a marker for the subsequent development of the oral injury.

Biography

Her research is focused on evaluating the molecular basis of DNA damage and repair pathways. Of particular importance in her current research is assessing the role of urokinase plasminogen activator in DNA repairing pathways associated with oral disease. Another line of her research involves the study of genetic and epigenetic influences of environmental toxicants. She has a Ph.D. in molecular biology from Hannover Medical School, Germany. Her Ph.D. research project entitled "Urokinase plasminogen activator receptor in DNA damage repair mechanism and senescence". She has also completed two postdoctoral research associate jobs, one at the Max Planck Institute of Immunobiology and epigenetics and one at the toxicology department, the Institute of pharmaceutical science, Tehran University of medical sciences.

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DNA recognition by the *BRCA1* tumor suppressor

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Statement of the Problem: Human *BRCA1* encodes a tumor suppressor protein that repairs double-stranded DNA breaks in cells. Mutations in *BRCA1* are closely linked to the early onset of breast and ovarian cancers. *BRCA1* protein participates in several DNA repair pathways but the molecular mechanisms through which *BRCA1* targets damaged DNA structures is not well-understood.

Methodology & Theoretical Orientation: A detailed comparison of the DNA binding preferences of *BRCA1* was performed on its DNA binding domains (DBD1 aa330-554, DBD2a aa894-1057, DBD2b aa936-1057, and BRCT aa1745-1861). Each *BRCA1* fragment was expressed in *E. coli* and purified from other proteins using nickel affinity and heparin ion exchange chromatography. The relative affinities of these purified *BRCA1* domains for various DNA targets (ssDNA, dsDNA, human G4 telomere, etc.) were measured by biolayer interferometry (BLI) as well as fluorescence spectroscopy. These equilibrium constant values were used to rank order the DNA binding preferences for each protein domain.

Findings: We find that DBD1 has the highest affinity for dsDNA. Both DBD2a and DBD2b show the highest affinity for single-stranded DNA, while BRCT binds tightest to the human G4 telomeric sequence.

Conclusion & Significance: The modular nature of these *BRCA1*-DNA interactions may provide a regulatory mechanism to control its DNA repair functions inside the cell. Therefore, we plan to perform DNA repair studies in human cell lines alongside these *in vitro* binding experiments to further test the link between DNA binding activity and repair of DNA lesions.

Biography

Ann J Fuelle is currently studying Biomedical Diagnostic and Therapeutic Sciences with concentrations in Medical Laboratory Sciences and Pre-Med studies. She started working in a laboratory at the University of Michigan in 2014 before attending Oakland University in 2016.

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Zhuoling He is currently studying Biochemistry with a Biology minor. Zhuoling hopes to obtain a PhD degree in the future. Both women work under the direction of Colin G. Wu at Oakland University in the field of Biochemistry studying DNA repair pathways.

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DNA damage repair response facilitated by the FANCJ Helicase and the REV1 polymerase

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Statement of the problem: G-quadruplexes (G4s) are DNA structures formed by guanine-rich nucleic acids. Damaged G4s can interfere with essential cellular processes such as DNA replication and RNA transcription. The human FANCJ helicase facilitates DNA replication through G4-forming regions and participates in interstrand DNA cross-link repair. G4s are also prone to oxidative stress and how such lesions are recognized and processed is not well-understood. REV1 is a translesion DNA polymerase that synthesizes DNA across from damaged templates in human cells. A current model states that REV1 is recruited to a G4 DNA site by the FANCJ helicase, and then participates in the repair process by incorporating cytosine across from a guanine base. In a previous study, we identified an AKKQ amino acid motif within FANCJ that binds to G4 DNA. FANCJ presumably targets a stalled replication fork at a G4-containing DNA site and then recruits REV1 to efficiently replicate DNA across from a G-quadruplex.

Methodology and theoretical orientation: In this work, we aim to test this model by examining the macromolecular interactions between REV1, G4 DNA, and the FANCJ helicase using fluorescence spectroscopy and biolayer interferometry.

Findings: FANCJ binds to REV1 with high affinity, which is consistent with this handoff model. In addition, we show that the FANCJ AKKQ motif also binds to an 8-oxoguanine modified G4, suggesting that FANCJ also recognizes damaged G4 structures.

Conclusion: Based on this evidence, FANCJ can target both G4 and 8-oxoG4 structures, and then bring REV1 to the DNA site in order to bypass the stalled replication fork. We plan to further examine the significance of the FANCJ-REV1 interaction in vivo by studying this G4 repair pathway in human cells.

Biography

Mena Jirjees is a Biochemistry student at Oakland University who is also pursuing a minor in Middle Eastern studies. She is currently applying to medical schools, and she is planning on becoming a neonatologist. Additionally, Mena would like to continue with basic science research in the field of cancer mechanisms.

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Kaitlin Lowran recently received her bachelor's degree in Biochemistry from Oakland University. She will begin her doctoral studies in the Biomedical Sciences program at OU starting this fall. She will be working with Dr. Colin Wu for her dissertation work. Kaitlin is interested in DNA repair mechanisms and she recently received a fellowship from the Michigan Space Grant Consortium to study these pathways in microgravity environments.

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Lysine acetylation of nuclear Pif1 alters its enzymatic function

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Petite integration frequency 1 (Pif1) is a 5'-3' helicase that is implicated in the maintenance of the fidelity of nuclear and mitochondrial DNA. The protein's preference for unwinding RNA-DNA hybrids makes it a key player during various DNA transactions in the cell such as DNA replication, repair, and telomere maintenance, where such substrates are abundant. We recently discovered Pif1 was post-translationally modified by lysine acetylation both in mammalian and *Saccharomyces cerevisiae* cells. The current focus of our work is to define the role of lysine acetylation in modulating the enzymatic properties of the Pif1. Genetic knockouts of the dynamic modifiers of lysine acetylation have implicated specific lysine acetyltransferases and deacetylases in the dynamic modification of Pif1. Using purified recombinant yeast acetyltransferases to in vitro acetylate full-length recombinant Pif1 protein, we tested the alterations in the enzymatic activities of acetylated Pif1 protein compared to the unmodified form. From the electromobility gel shift assays (EMSA) and biolayer interferometry (BLITZ) analysis, we found that the acetylated form of Pif1 had higher binding affinity compared to the unmodified form. Directly correlating to increased substrate binding, the acetylated form of Pif1 also showed significantly higher helicase and ATPase activity. Additionally, using mass spectrometry, we have mapped out the sites of lysine acetylation of both in vivo and in vitro acetylated Pif1. We are currently in the process of understanding specific cellular triggers of Pif1 acetylation and also the impact of lysine acetylation of Pif1 on genetic stability.

Biography

Onyekachi Ononye is a graduate student in Dr Balakrishnan's laboratory at Indiana University-Purdue University Indianapolis. Her research focus explores the role of lysine acetylation in modulating DNA replication proteins on the lagging strand. Using a plethora of biochemistry techniques, Onyekachi is working towards defining one of the regulatory pathways required for high fidelity processing of DNA.

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Nucleolin functions as a negative regulator of androgen receptor transcription

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Androgen receptor (AR) drives the development and progression of prostate cancer (PCa). Men who develop regionally advanced or metastatic prostate cancer often have long-term cancer control when treated with androgen-deprivation therapies (ADT), but their disease inevitably becomes resistant to ADT and progresses to castration-resistant prostate cancer (CRPC). ADT involves the use of potent competitive AR antagonists and androgen synthesis inhibitors. Resistance to these treatments often emerges through maintenance of AR signaling via ligand-independent activation mechanisms. There is a need to identify the molecular mechanism that regulates the AR signaling expression to develop novel therapies that enhance the efficacy of existing systemic therapies for CRPC patients. Here, we present evidence that implicates nucleolin (NCL) as a regulator of AR expression. The promoter of AR contains G-rich sequences that can form G-quadruplex structures (G4). We found that NCL binds to this G4 region within the AR promoter. A dual reporter assay showed that genetic knockdown of NCL increases the transcription activity of AR promoter only when the AR promoter G4 sequence is present. Genetic knockdown of NCL also increases the levels of both AR mRNA and protein in PCa cells but has no effect on AR mRNA stability. The ability of NCL to modulate AR expression was independent of AR activation. Moreover, compounds that stabilize G4 structures and increase NCL association with the G4 of the AR promoter decrease AR expression. These results indicate that NCL functions as a transcriptional repressor of the AR gene, and raise the important possibility that G4-stabilizing drugs can increase NCL transcriptional repressor activity to block AR expression. These findings contribute to a clearer understanding of the mechanisms that control the expression of AR and may be of significance for the development of alternative therapeutic options for men with CRPC.

Biography

Elsa Reyes-Reyes has been working in the field of cancer biology for about 14 years. Her research focus has been to define signaling pathways that promote cancer progression using *in vitro* and *in vivo* models. Her research goal is to characterize and identify therapeutic targets with high clinical application potential for the treatment, diagnosis, and prognosis of cancer. During the past decade, she has acquired solid experience in drug development for the treatment of different types of cancer such as colon, lung, liver, and prostate. My research has led me to be co-inventor on five patents.

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Kinetic basis for DNA target specificity of CRISPR-Cas12a

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Class 2 CRISPR-Cas nucleases have become the gold standard for genome editing, as genetic manipulation can be achieved by a relatively simple and easily adaptable system1. The common class 2 CRISPR-Cas nucleases, Cas9 and Cas12a (previously known as Cpf1), are programmable via a guide RNA that uses complementarity with DNA to identify the correct target. Still, cleavage at off-target sites resembling the intended sequence remains a pervasive problem2. DNA targeting by Cas12a is more specific than Cas93, but the mechanistic basis for this increased specificity is not understood. To dissect the reaction steps of DNA targeting by Acidaminococcus sp Cas12a, we used a quantitative kinetics approach, measuring rate constants for binding, dissociation, and DNA strand cleavage. We show that Cas12a binds DNA in two kinetically-separable steps; protospacer-adjacent motif (PAM) recognition is followed by R-loop formation. Once it is bound, Cas12a cuts the non-target strand repeatedly, trimming it back towards the PAM, and then it cleaves the target strand imprecisely. Because R-loop formation is rate-limiting for cleavage, Cas12a binding inevitably leads to target DNA cleavage. Even when targeted to DNA with single mismatches, Cas12a binding remains rate-limiting for DNA cleavage. Thus, the specificity of Cas12a for the matched target is defined by the kinetics of binding rather than by affinity. Nevertheless, in contrast to simple expectations for R-loop formation, we show that Cas12a retains substantial specificity against mismatches throughout the R-loop. This strong discrimination indicates a late transition state for binding, implying that R-loop propagation is reversible up until the R-loop has almost entirely formed. Our results provide a mechanistic foundation for the DNA cleavage patterns of Cas12a measured in vivo 3-5 and lead to a model for the higher specificity of Cas12a than Cas9.

Biography

Isabel Strohkendl has a strong curiosity for understanding the mechanisms by which proteins with diverse structures and functions act on DNA. Under the supervision of Dr Rick Russell at the University of Texas at Austin, she has been studying protein-nucleic acid interactions and using quantitative biophysical approaches to elucidate the mechanisms of DNA-binding enzymes. Currently, her work focuses on understanding how both nucleic acid and nucleic acid-protein interactions dictate substrate preference during Cas12a-crRNA assembly and contribute to Cas12a specificity during DNA targeting.

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Effect of Dioclea grandiflora DGL-II lectin on human epidermal keratinocyte proliferation and migration: Relationship with its glycotope specificities

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Statement of problem: Impaired or delayed re-epithelialization during wound healing represents a medical problem in many organs. Keratinocyte migration and proliferation are the most relevant events in skin re-epithelization. The present study purpose was to observe the effect of DGL-II on the proliferation and migration in human epidermal keratinocyte cell line HaCat; likewise, on the combined approach basis, to make a parallel of similarities and disparities between glycotope ligands for this plant lectin with those for mammalian galectin-3 (LGALS3), taking into account that the latter promotes re-epithelialization under natural and in vitro conditions.

Methodology & Theoretical Orientation: DGL-II purification was carried out by lactose affinity as previously described. MTT, trypan blue dye exclusion viability and cell migration (scratch) assays were performed in presence of DGL-II. A comparison between ligands for DGL-II and for LGALS3 was made.

Findings: DGL-II showed a pro-proliferative effect in the same concentration range as did LGALS3, as well as, it increased the rate of scratch width closure in the monolayer compared to the control. DGL-I, another lectin from the same plant but with different specificities, did not. DGL-II and LGALS3 recognize structures containing the same glycotopes (e.g. IIβ and Neuα2,3Gal). Exposed α-linked galactose and fucosylation can affect interactions. Some ligands shared by DGL-II and LGALS3 are part of membrane glycoconjugate structures involved in proliferation and migration of molecular signaling.

Conclusion & Significance: DGL-II increased keratinocyte proliferation and migration rate within the range of concentrations in which exogenous LGALS3 did it, and shows similar specificities to this mammal galectin. This makes us think that DGL-II could increase the rate of closure and recovery of skin lesions. We have the perspective to observe the DGL-II effect on the expression of molecules involved in cell proliferation and migration, as well as to evaluate it as a skin treatment in mammal model.

Biography

The main contributions I have provided during my career and my research has to do with that organ that surrounds and covers us like we were gifts: Our skin. I had the opportunity to investigate about the host-parasite relationship, changes at the cellular level and mechanisms of action of drugs and vaccines against a disease that produces skin ulcers: cutaneous leishmaniasis. Then, I was working for a company for skin care products where I could evaluate in vivo and in vitro efficacy and safety of skin products. Currently, I am developing my doctoral thesis "Effect of plant lectins on proliferation and migration processes in epidermal keratinocytes", at the National University of Colombia.

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N-Glycosylation type and structure analysis of HIV-1 and Anti-HIV-1 broadly neutralizing antibodies (bnabs) by sequential Exoglycosidase cleavage and Hilic Chromatography

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Oligosaccharides in glycoproteins influence many aspects of protein function, e.g. half-life, potency, immunogenicity, efficacy etc. Regulatory agencies require demonstration of consistency in glycosylation of the manufactured lots for human therapy. Therefore, characterization of carbohydrate is necessary for therapeutic and preventative glycoproteins. In this poster, N-glycans of an HIV-1-envelope (Env) protein vaccine candidate and five HIV-1 broadly neutralizing antibodies (bNAbs) have been characterized by means of Endoglycosidase deglycosylation and followed by exoglycosidase sequencing of oligosaccharides to give structural information on the sequence of monosaccharides and type of linkage within the oligosaccharide chain. From the data of sequential treatment of the panel of eight exoglycosidases, and the known specificity of the exoglycosidases, the type, order and linkage of monosaccharide within the N-glycan chain were deduced. The N-glycan species were separated by hydrophilic interaction chromatography (HILIC) and the glycan peaks were identified. Given the facts that the HIV-1-envelope (Env) trimer protein is covered by a glycan shield of 84 (3x28) N-linked oligosaccharides, and the five HIV-1 bNAbs have N-glycosylation sites in either Fc region or in both Fab and Fc region, which is challenging for glycan analysis. The successful utilization of endoglycosidase (PNGaseF) and exoglycosidase panels give unique characteristic N-glycan profiles for the HIV-1 trimer vaccine and the five bNAbs. The results (Figure 1) demonstrate (1) predominant high-mannose species in the HIV-1; (2) neutral complex species in 10E8; (3) neutral and afucosylated sialidated complex in N6 and VRC07; (4) G0f is the predominant glycoform in VRC01 along with the other neutral and fucosylated/afucosylated sialidated complex species; (5) fucosylated/sialidated and neutral complex glycoforms were found for CAP256. Although mass spectrometric methods can provide accurate masses of molecular or fragment ions, they are unable to distinguish between isomeric monosaccharide residues (e.g. all neutral hexoses have the same mass of 162 Da). In such case, exoglycosidase with a known highly specific sequential degradation can provide fast and accurate oligosaccharide linkage identification for HIV-1 vaccine and bNAbs development.

Biography

Dr Yanhong Yang has her expertise in protein characterization utilizing LC, LC/MS, and electrophoresis techniques. She had been working in the biopharmaceutical industry for over ten years focusing on recombinant protein characterization including protein carbohydrate analysis, primary and secondary structure characterization (Amino Acid sequencing, PTM, disulfide bond linkage etc.). She also has experience in analytical project representative role for Phase 1 to Phase 3 project development. In November 2016, Dr Yang joined Vaccine Product Production in Vaccine Research Center of NIH. She has been working on HIV Trimer and HIV neutralizing mAbs from research candidate to Phase 1 clinical trial, focusing on analytical assay development for product quality attribute monitoring, including aggregates, product purity, charge heterogeneity and N-glycosylation etc.

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Mechanistic investigations of α-glucosidase activity

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Emergent BioSolutions is currently developing antivirals with an iminosugar structural motif that targets the endoplasmic reticulum (ER) alpha-glucosidase enzymes. The mechanism of action is based on the inhibition of host glycosylation pathway that leads to misfolded viral glycoproteins, resulting in reduced viral infectivity. Data generated by Emergent and others indicate that iminosugars have the potential to be developed as a treatment for diseases such as Dengue Fever, influenza, Ebola, and Zika. To support our drug discovery efforts, preliminary mechanistic investigations into the protein synthesis and folding pathway have been initiated. We have verified the impact of inhibition on α-glucosidase activity by showing inhibition of viral proliferation in human cell lines knocked out for each of the ER glucosidases using CRISPR-Cas9. With collaborators at the Oxford Glycobiology Institute and a commercial MALDI-MS vendor, we evaluated in vitro glycan profiling in the CRISPR cells by both a chemical derivatization/HPLC method and by MALDI-MS of permethylated glycans. These orthogonal techniques confirm that knock-out of alpha-glucosidase 1 and alpha-glucosidase 2 enzymes prevents the cleavage of the terminal glucose units involved in glycoprotein maturation. Examining the changes in glycan product distribution after treatment of cell extracts with alpha-glucosidase 1 reveals the loss of a single glucose, while treatment with alpha-glucosidase 1 and alpha-glucosidase 2 results in the loss of two and three glucose units. These techniques can be used to confirm the mechanism of action of new chemical entities from our iminosugar med-chem program. Work described here was performed in collaboration with Anthony Treston and Kelly Warfield of Emergent, Dom Alonzi and Nicole Zitzmann of Oxford University and Craig Day of Utah State University.

Biography

Daniel C Hill is a PhD chemist with 20 years' experience in the pharmaceutical industry solving complex problems in results-driven departments working on programs in early discovery supporting candidate selection, drug development and on through to marketed commercial products. Dan has diverse experience in a variety of therapy areas along the critical path of drug discovery and development including Neurokinin Receptor Antagonists, gamma-Secretase, 5HT-1b Receptor Antagonists, Gap Junction Modulators, Topoisomerase Inhibitors, and Iminosugar alpha-Glucosidase Inhibitors.

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Biochemical and structural characterization of STARD10, a phospholipid binding protein

Ekaterina Shishova & John Gonzales
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Phospholipids are the major structural components of biological membranes and mediators of signal transduction. In cells, these water insoluble molecules are exchanged between membranes via a group of transport proteins known as steroidogenic acute regulatory protein–related transfer (START) domain superfamily of proteins. Proteins containing START domain function in many physiological processes, such as lipid transfer between intracellular compartments, lipid metabolism, and cell signaling events. One such lipid transporter is StARD10, a 33 kDa protein, originally identified as a protein overexpressed in breast cancer. While its physiological role is still not clearly understood, studies suggests that StARD10 is highly expressed in liver and the mammary gland, may be used as a biomarker for cancer, and it is also required for normal insulin secretion. Closely related to StARD2, a phosphatidylcholine transfer protein, StARD10 was reported to bind and transfer both phosphatidylcholine and phosphatidylethanolamine. Here, we report a study in which recombinant human StARD10 was purified and tested for phospholipid transfer activity using an in vitro fluorescence quench assay for intermembrane transfer of 7-nitro-2,1,3-benzoxadiazol-4-yl (NBD) labeled phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. The protein showed higher transfer activity for a phosphatidylcholine with NBD labeled head group, followed by about four fold reduced activity for head group labeled phosphatidylethanolamine, and only a negligent transfer activity for a tail labeled phosphatidylcholine. There was no transfer activity observed for phosphatidylserine. Through analysis of closely related phosphatidylcholine transfer protein StarD2 three dimensional structure and primary sequence alignment with StARD10, the key phospholipid binding amino acids evolutionary conserved in both StARD2 and StARD10 were identified. Further, 3-dimensional homology models of StARD10 without and with phospholipid substrates were generated by SWISS MODEL and SWISS Dock, respectively, and provide a useful insight into the substrate binding mode in StARD10 active site.

Biography

Ekaterina Shishova is an Assistant Professor of Biochemistry at the Sage Colleges in Troy, NY. She has expertise in structural biochemistry. She hold a Ph.D. in chemistry from the University of Pennsylvania where she completed research on X-ray crystallographic studies of metallo-enzymes. Further, she received post-doctoral training at Brigham and Women's Hospital and Harvard Medical School where she worked on biochemical characterization and development of small molecule inhibitors of lipid binding proteins.

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Dynamic aspects of the half-life of drugs, preclinical biomarkers-un update

Admir Nake, and **Elizana Petrela**
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Introduction: Half-life of drugs, useful substances(lab exams, calibrators, biomarkers), after the validation process, have the great application status in medical practice. Very important achieving to modify half-life of these "important substances" is direct contributors to the efficacy of treatment, different clinical decision and improving life or quality of life of the patient and more.

Aim: Importance and feature of the half-life of drugs, lab exams, reagent and calibrators, and ways to modify and affect that. We should know main "pits stops" affected half-life of drugs, functional group role, steric effect, polarization of molecules, (as if voltaren vs fenoprofen, carbamazepine vs oxcarbamazepine, testosterone vs methyltestosterone), interacting factors, responsible events (microbiome and clearance role), responsible organs (liver, kidney etc.), and importance of ways to modify half-life of them. Half-life of circulating substances is important to select biomarkers such as for myocardial infarction(ischemia modified albumin, myoglobin, CK, CK-MB, troponin, LDH), inflammatory biomarkers (soluble fraction of CD14, pentraxin-3, procalcitonin, C-reactive protein) indicators of nutritional status(retinol binding protein, prealbumin, transferrin, albumin). And glycosylated NT-proBNP (natriuretic peptide) as calibrator.

Conclusion: Biochemical events or "biochemical intervention" (conjugation, lipidation, PEG and HES methods, albumedix, cell penetration peptides or dendrimers), modify features of drugs and lab or imagery biomarkers and the ways of their applications. They've broken taboo of "static frame" of these important substances", and are very useful "window", have brought new useful discoveries, new drugs, new lab and imagery biomarkers or methods as direct contributors in efficacy of treatment, different clinical decision and improving life or quality of life of the patient and more.

Biography

Expertise in lab, medicine, especially microscopy, imunoenzymology, biochemistry exams, biomarkers, lab as a part of public health policy in Albania, improving and updating point of view in diagnosis, screening and prognosis criteria, education and curricula of lab, tech staff, environment projects and application of principles of Aarhus convention.

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Notes:

1952nd Conference

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5th International Conference on

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&

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Accepted Abstracts

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Association of triglycerides to HDL ratio as a marker to detect cardiovascular risk factors among adults in a Tertiary care hospital setting Pakistan

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This study conducted with the aim to find an association of Triglycerides to HDL ratio in adults with cardiovascular risk factors (Obesity, diabetes, and hypertension).study design was a Cross-sectional study conducted in Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology Rawalpindi from January 2018-June 2018. This study was a cross-sectional study conducted in Armed forces institute of Pathology CMH Rawalpindi. Inclusion criteria include an adult with a range of 19-50 year of age. Patient with comorbidity like cancer, tuberculosis, bedridden patient will be excluded from the study. Sampling Technique is simple random sampling. A structured; standardized and pre-tested questionnaire was applied in a pilot study. The independent variables evaluated at the first meeting will be, socioeconomic and demographic (age, gender, marital status, years of schooling and economic class), and presence of morbidities (heart disease, dyslipidemia, hypertension, diabetes, the presence of depressive symptoms). A total of 355 sample size was analyzed after getting approval from an ethical review board of Armed forces institute of pathology CMH Rawalpindi, out of which 269(71.5%) were female while 86(22.9%)were male with mean age of 37±11.64 year with a range of 22-60 year of age group. To check association among cardiometabolic Risk factors like Diabetes, obesity, hypertension and TG/HDL-C ratio chi-square test computed for all cardiometabolic Risk factors. It shows strong association among HOMA-IR, Diabetes, Hypertension, percent body fats and TG/HDL-C ratio (p= 0.00, p=0.001.p=0.00,p=0.00).TG/HDLC ratio considered as a potential biomarker for the early prediction of cardiometabolic risk factors.

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Glycosyl cation: From observation to mimicry

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Glycosyl cations are crucial intermediates formed during enzymatic and chemical glycosidic bond formation and hydrolysis. The high reactivity and short lifetime of these reaction intermediates make them very challenging to characterize using spectroscopic techniques. Their observation is thus of high current interest to both gain insight into the mechanistic pathways of SN1-like glycosylation at the atomic level and to design potent inhibitors of the glycoside processing enzymes that go through such cationic intermediates by mimicry. Using super acidic conditions, we have been able to recently observe such chemical species is their polyprotonated form in a condensed phase and to have access to their confirmation. Our most recent results dealing with this approach will be presented as well as our parallel efforts to design potent glycosidase inhibitors mimicking such cationic intermediates in order to target enzymes of therapeutic interest.

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Molecular evolutionary relationships between O antigens of enteric bacteria

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Statement of the Problem: Enteric bacteria *Escherichia coli* is the predominant facultative anaerobe of the colonic flora, and some specific serotypes are associated with enteritis, hemorrhagic colitis, and hemolytic uremic syndrome. *Shigella* spp. are human pathogens that cause diarrhea and bacillary dysentery (shigellosis). Strains of *Salmonella enterica* are responsible for a food-borne infection (salmonellosis), and specific serotypes cause typhoid fever and paratyphoid fever. Salmonella and *Escherichia* diverged about 140 million years ago. *Shigella* spp. evolved 150 to 300 years ago and are in effect *E. coli* with a specific mode of pathogenicity. All these bacteria are closely related with respect to structure and genetics of the lipopolysaccharide, including the O-polysaccharide part (O antigen). Being exposed to the bacterial cell surface, the O-antigen is subject to intense selection by the host immune system and bacteriophages giving rise to diverse O antigen forms and providing the basis for typing of bacteria. The O-antigen forms of many bacteria are unique, but some are structurally and genetically related to others.

Methodology & Theoretical Orientation: The sequenced O-antigen gene clusters between conserved galF and genes were analyzed taking into account the O-antigen structures established by us and others for all *S. enterica* and *Shigella* and most *E. coli* O-serogroups. Multiple genetic mechanisms of diversification of the O-antigen forms, such as lateral gene transfer and mutations, were elucidated and are summarized in the present paper. They include acquisition or inactivation of genes for sugar synthesis or transfer or recombination of O-antigen gene clusters or their parts.

Conclusion & Significance: The data obtained contribute to our understanding of the origins of the O-antigen diversity, shed light on molecular evolutionary relationships between the O-antigens of enteric bacteria, and open a way for studies of the role of gene polymorphism in pathogenicity.

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The structural basis for the interdependence of drug resistance: HIV-1 protease

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HIV-1 protease is responsible for the cleavage of 12 non-homologous sites within the Gag and Gag-Pro-Pol polyproteins in the viral genome. Under the selective pressure of protease inhibition, the virus evolves mutations within (primary) and outside of (secondary) the active site, allowing the protease to process substrates while simultaneously countering inhibition. The primary protease mutations impede inhibitor binding directly, while the secondary mutations are considered accessory mutations that compensate for a loss in fitness. However, the role of secondary mutations in conferring drug resistance remains a largely unresolved topic. We have shown previously that mutations distal to the active site are able to perturb binding of darunavir (DRV) via the protein's internal hydrogen-bonding network. In this study, we show that mutations distal to the active site, regardless of context, can play an interdependent role in drug resistance. Applying eigenvalue decomposition to collections of hydrogen bonding and van der Waals interactions from a series of molecular dynamics simulations of 15 diverse HIV-1 protease variants, we identify sites in the protease where amino acid substitutions lead to perturbations in nonbonded interactions with DRV and/or the hydrogen-bonding network of the protease itself. While primary mutations are known to drive resistance in HIV-1 protease, these findings delineate the significant contributions of accessory mutations to resistance. Identifying the variable positions in the protease that have the greatest impact on drug resistance may aid in the future structure-based design of inhibitors.

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