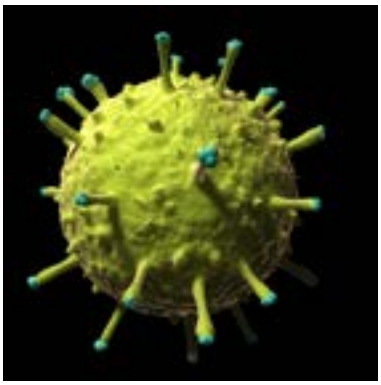
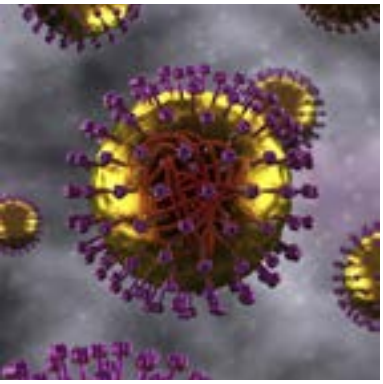
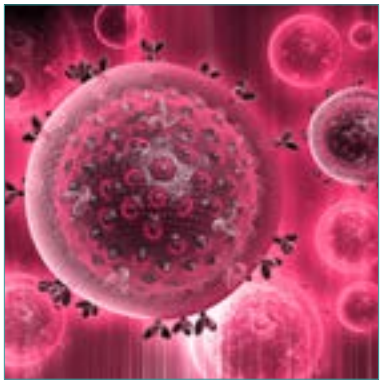
Scientific Tracks & Abstracts
October 30, 2017

Virology Conference 2017







International Virology Conference

October 30-31, 2017 | Toronto, Canada

Novel therapeutics for HIV-1: Small molecule modulators of RNA processing

A Cochrane[1], R Wong[1], A Balachandran[1], L Liu[1], T Cabral[1], D Shen[1] and **P Stoilov[2]**
[1]University of Toronto, Canada
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Current therapeutics is highly successful in blocking disease progression following HIV-1 infection but the development of resistance due to the high mutation rate of this virus remains a constant threat. To explore alternative approaches of controlling HIV-1 replication that would complement existing drugs, we are examining the impact of altering various processes regulating host RNA splicing for their ability to modulate HIV-1 gene expression. Previously, our group demonstrated that cardiotonic steroids (digoxin) are potent inhibitors of HIV-1 replication due to alterations in viral RNA processing associated with the selective modification of the host RNA processing factors SRSF3 and Tra2ß. In more recent work, we demonstrate that the anti-HIV-1 effect of digoxin is the result of MEK/ERK signaling pathway activation. In support of the importance of this pathway in regulating HIV-1 RNA processing, parallel studies identified other activators of this pathway (anisomycin and a benzoxadiazol-4-amine derivative designated 191) as potent inhibitors of HIV-1 gene expression. Like digoxin, 191 induced a marked alteration of HIV-1 RNA processing (reduced accumulation of unspliced and singly spliced viral RNAs) as well as loss of Tat and Rev Expression and changes in SRSF3 and Tra2ß phosphorylation. At doses of 191 sufficient to suppress HIV-1 replication, we observe only minor changes in host RNA alternative splicing (<30 events showing >20% alteration in exon inclusion of 9800 events detected) consistent with HIV-1 having a great sensitivity to modulation of the host RNA splicing apparatus. Subsequent tests have confirmed 191's ability to suppress HIV-1 replication in the context of PBMCs. Furthermore, 191 are able to inhibit replication of different HIV-1 clades as well as variants resistant to existing RT, PR, or IN inhibitors. In parallel with the above studies, we have also examined the effect of modulating the activity of other host cell signaling cascades (AKT, PI3K, GSK-3) for their effects on HIV-1 gene expression. Of these pathways, only inhibition of GSK-3 with CHIR98014 was found to result in loss of viral protein expression that correlated with reduced HIV-1 RNA accumulation. CHIR98014 is a potent inhibitor of HIV-1 gene expression in all cell lines tested and is currently being evaluated for its anti-viral potency in the context of PBMCs. Current tests have not detected changes in host SR protein phosphorylation/abundance that correlate with CIHR98014's anti-HIV effect. However, shRNA depletion of either GSK-3α or ß resulted in a loss of HIV-1 Gag expression confirming the important role of this signaling pathway in regulating HIV-1 gene expression. Together, these findings demonstrate the feasibility of modulating host RNA processing to generate a state within the cell unable to support HIV-1 replication.

Speaker Biography

A Cochrane completed his PhD in 1988 from Queen's University and Post-doctoral studies from Roche Institute of Molecular Biology. He is a Professor at the University of Toronto. He has published more than 70 papers and serving on the Editorial Board of Retrovirology. Over the last several years, his research has been focused on the regulation of viral RNA processing, with particular focus on the identification of small molecule modulators of RNA splicing and their utility in the suppression of HIV-1 and adenovirus replication.

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Notes:

Noncytolytic destruction of dsDNA viral episomes: Anti-HPV agents for prevention of cervical and other cancers which modulate the DNA damage response and are also active against polyomaviruses

James K Bashkin
Oxford University, UK

Our program on anti-human papillomavirus (HPV) compounds was inspired by Dervan and Sugiyama's work with hairpin pyrrole-imidazole polyamides, but nothing we believed initially turned out to be true even though we discovered very active compounds. We initially targeted the long control region of the doubled-stranded, circular, negatively super-coiled DNA genome of HPV, hoping to block binding of viral proteins necessary for replication. We made large polyamides in an attempt to minimize their accessibility to human chromatin, and this size turned out to be important because activity was observed only for polyamides that bound at least one full turn of B-form DNA. However, it was immediately obvious that our active molecules were more powerful than theoretically possible for replication inhibitors. In fact, after further experimentation, we found that antiviral polyamides were causing active degradation of viral DNA. We were surprised to discover broad spectrum activity against HPV16, 18 and 31, all oncogenic strains, given the reported rules for polyamide-DNA recognition on which our structures were originally based. More recently, we conducted preclinical safety studies on lead and backup compounds, discovered a new mechanism of action for polyamides and antivirals in which the DNA Damage Response is activated and found that our compounds fail to follow reported polyamide-DNA binding rules. We discovered various guanidinium N-termini that improved antiviral activity, and we embarked on massively parallel sequencing-based studies to further probe the mechanism of action. Antiviral results were also extended to other small DNA tumor viruses, the polyomaviruses SV40, BKV-Dun, and BKV-TU.

Speaker Biography

James K Bashkin completed his DPhil from Oxford University (UK) and Post-doctoral studies from Harvard University. He is the Professor of Chemistry and Biochemistry at the University of Missouri- St. Louis and is the Director of Chemistry at NanoVir, a company he co-founded. He has published more than 70 papers in reputed journals, 15 issued US patents, and served as an associate editor and Editorial Board Member for the Royal Society of Chemistry and American Chemical Society. He received the Thomas and Hochwalt Prize (1994), Presidential Green Chemistry Challenge Award (1998), and Roland Tibbetts Award (2006).

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Notes:

Characterization of the entry mechanism of a novel protein transduction domain originated from Betanodavirus

Junfeng Xie, Guohua Zhu, Jing Zhang and Runqing Huang
Sun Yat-Sen University, China

Betanodavirus, also called nervous necrosis virus (NNV), is the causative agent of a fish disease viral nervous necrosis. VNN causes high mortality in marine fish at larvae and juvenile stages resulting in heavy financial losses of marine aquaculture industry. Base on the sequence analysis and 3D structure elucidation of orange-spotted grouper nervous necrosis virus (OGNNV), we found a peptide with the capsid protein can carry foreign protein to enter fish cells. Through cell entry experiment with different sections of the peptide fused with GFP, we characterized a novel 14-aa peptide as protein transduction domain (PTD) and named NNV-PTD. NNV-PTD can be fused with foreign protein at N- or C-terminal without disturbing the entry efficiency. In addition, NNV-PTD can enter different types of fish cells, insect cells and mammalian cells as well as enters fish cells with higher efficiency than well-known PTD such as TAT, Penetratin and R8. NNV-PTD entered fish cells to perinuclear regions within 4 h and can be blocked by culturing at 4, indicating the endocytosis-dependent entry mechanism. Therefore, we used the biochemical inhibitors blocking clathrin-mediated endocytosis (CME), micropinocytosis, caveolae-dependent endocytosis, cellular cholesterol, low-pH balance and cytoskeleton to treat cells before entry assay. Finally, we demonstrated that NNV-PTD enters fish cells via CME depending on dynamin and macropinocytosis depending on myosin α, and also the entry is cholesterol-, low-pH, and cytoskeleton-denpendent at the intracellular traffic level. Together, this work not only characterizes a novel high efficient PTD but also identifies its entry mechanism, providing basic information for further application of NNV-PTD in aquaculture.

Speaker Biography

Junfeng Xie has completed his PhD from Sun Yat-sen University, China. He is the Associate Professor of School of Lifesciences, Sun Yat-sen University, USA. He is focusing on the basic virology research of Betanodavirus and on the application study of antiviral agents.

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Notes:

Feeding kelp meal improved kidney function of mink challenged with the Aleutian Mink disease virus

A H Farid[1] and **N J Smith**[2]
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Aleutian mink disease virus (AMDV) is endemic in Nova Scotia (NS), Canada, and causes considerable economic losses to the industry. Failure of virus eradication from many farms in NS after more than 30 years of test-and-removal strategy forced many farmers in this province to select their herds for tolerance to AMDV. Mink herds which are under selection for tolerance show high mortality and reduced performance for several years, and any feed additive or pharmaceutical that can ease the negative effects of infection are of particular interest to mink farmers. The objective of this study was to investigate the effects of feeding the brown algae Ascophylum nodosum (kelp meal) on serum parameters of mink infected with AMDV. A total of 75 AMDV-free female black mink were inoculated intranasally with a spleen homogenate containing a local strain of the virus. Mink were fed a commercial pellet with the kelp meal added at the rates of 0% (control), 0.75% and 1.5% of the feed. Animals were killed after 451 days of feeding kelp, and serum samples were collected. Serum total proteins, albumin, alkaline phosphatase (ALKP), urea nitrogen, creatine, globulins and gamma-glutamyl transferase (GGT) were measured using the Vet-Test Chemistry Analyzer (IDEXX International). Data deviated from normality and treatment effects were compared by the non-parametric Kruskal-Wallis test. In cases where this test was significant at $\alpha<0.05$, pairwise comparison of treatment means was performed by the Mann-Whitney U test and Bonferroni correction. Feeding kelp had a significant effect only on urea nitrogen and creatine, which were significantly higher in the control group than in the 1.5% kelp. Urea nitrogen and creatine were intermediate in mink that were fed 0.75% kelp and was not different from the other groups. The results suggested that feeding 1.5% kelp significantly improved the kidney function. Improved animal health through improved kidney function, independent of changes in serum proteins, is of considerable importance when selecting for tolerance.

Speaker Biography

A H Farid is an Adjunct Professor in the Department of Animal Science and Aquaculture at Dalhousie University Faculty of Agriculture. He received his PhD degree in Animal Breeding and Genetics from the University of Alberta in 1986. He joined Dalhousie University in 1990 and retired in 2017. His research has been focused on the application of molecular techniques to animal improvement, including genotyping of Canadian purebred sheep for resistance to scrapie, and genetic selection of mink for resistance to the Aleutian mink disease virus. He has written one book chapter, published 77 papers in refereed scientific journals and more than 250 abstracts, technical papers and presentations to the livestock industries.

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Notes:

Determinant of receptor-preference switch in influenza hemagglutinin

Qinghua Wang[1], **Fengyun Ni**[1] and **Elena Kondrashkina**[2]
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[2]Northwestern University, USA

Hemagglutinin (HA) is one of the two major glycoproteins on the surface of influenza virus. One main function of HA is to selectively bind to sialic-acid receptors on host cells to trigger viral entry by endocytosis. There are two types of sialic-acid receptors that HA recognizes: (2,3)-linked avian-like receptors and (2,6)-linked human-like receptors. Frequently, a small number of substitutions in HA would endorse a switch in receptor-binding specificity from avian-like to human-like receptors, thus allowing cross-species transmission. However, the set of residues required for such a receptor-binding specificity switch differs among various subtypes of influenza type A virus. In my talk, I will discuss the results of our most recent study in understanding the underlying principles of this process.

Speaker Biography

Qinghua Wang has completed her Bachelor's degree at Peking University in China and PhD degree at University of Cambridge in Britain. She then received Post-doctoral training at Harvard University. She is an Assistant Professor at Baylor College of Medicine, Houston, Texas, USA. Her laboratory has made seminal contributions to our understanding of influenza type A and type B virus hemagglutinin.

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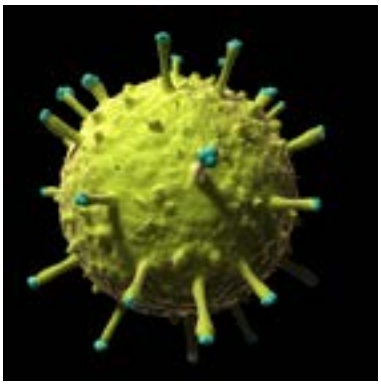

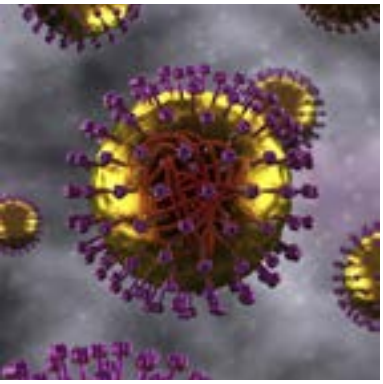

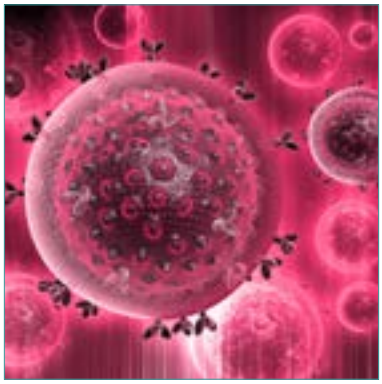

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Quantitative assessment of severity of histopathological lesions in mink infected with Aleutian mink disease virus

R Khomayezi and **A H Farid**
Dalhousie University, Canada

Mink production is one of the most important agricultural industries in Nova Scotia, the province with the greatest number of mink pelts produced in Canada. Aleutian mink disease virus (AMDV) causes Aleutian disease (AD) that seriously threatens this industry. The main characteristics of AD in adult mink are plasmacytosis (abnormally high number of infiltrated plasma cells in tissues), viremia, hypergammaglobulinemia, high anti-AMDV antibody titers and presence of circulating infectious immune complexes. Severity of histopathological lesions of AD is often subjectively assessed under a light microscope; thus, the results are not comparable among laboratories. The objective of this study was to develop a quantitative method of measuring the severity of histopathological lesions in AMDV-infected mink using a digital image analysis system. Slides were prepared from the kidneys of 5 infected mink and stained with hematoxylin and eosin. Three images were taken from different areas of each slide and were transferred to image analysis software, Image Pro Plus v7. Infiltrated plasma cells could not be accurately identified by automatic feature of the software, hence were counted using its manual feature by clicking on each of the intended cells and adding them to a list of counted objects. For calculating the percentage of infiltrated plasma cells, the number of total cells on each image was counted by the automatic feature of the software. The software detects the nuclei of cells based on the color intensity (gray scale) of their constituted pixels. Therefore, it was necessary to find a range of color intensity by which the software could correctly count the largest number of nuclei. For comparison, total cells were counted visually and by the software on one randomly chosen area on 15 kidney images. Sensitivity and precision of the software in counting total cells in each of the ranges of gray scale (0 and 255) within the three color-channels (red, blue, green) were calculated. The highest sensitivity (0.95) and precision (0.99) were achieved in the red channel. The averages of sensitivity and precision at each of the ranges of gray scale in the red channel were calculated for the 15 images, which were high at the rage of 0 to 90. This range of gray scale was used to count the total number of cells in kidney slides of AMDV-infected mink.

Speaker Biography

R Khomayezi has completed his Doctor of Veterinary Medice (DVM) degree from Azad University of Tabriz and then worked as a Practitioner in his own veterinary clinic for three years. He was admitted in a research-based Master of Animal Science program at Dalhousie University in 2015. His research project is focused on finding relationships between the degree of histopathological lesions and serological changes in AMDV-(Aleutian mink disease virus) infected mink. Passionate about veterinary pathology and virology, his research project has been a great fit. He is planing to defend his thesis by the end of April 2018.

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Pattern recognition receptor-initiated innate antiviral response in adipocytes

Lili Yu
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Adipose tissue had long been considered as a site that stores energy. Although wide range of viruses can infect adipose tissues, innate antiviral response of adipose cells has not been investigated. Adipose cells are equipped with innate antiviral system. Major virus sensors including Toll-like receptor 3 (TLR3), melanoma differentiation-associated antigen 5 (MDA5) and retinoic acid-inducible gene I (RIG-I) are constitutively expressed in preadipocytes and adipocytes. Poly(I:C), a common agonist of TLR3, MDA5 and RIG-I, induced the expression of type I interferons in the two types of adipose cells through the activation of IFN-regulatory factor 3 and upregulated proinflammatory factors such as TNF-α and IL-6 through the activation nuclear factor kappa B. Cytosolic DNA sensor p204 and its signaling adaptor stimulator of interferon (IFN) genes (STING) were constitutively expressed in adipocytes. Synthetic herpes simplex viral DNA (HSV60), a p204 ligand, induced type I IFN expression by activating IFN regulatory factor 3. Many major antiviral proteins, including IFN-stimulating gene 15, 2'5'-oligoadenylate synthetase and Mx GTPase 1 could be activated by both poly(I:C) and HSV60. poly(I:C) inhibited preadipocyte differentiation in a dose-dependent manner, but not in a time-dependent manner. Endogenously transfected poly (I:C) severely impaired the adipogenesis of preadipocytes compared with exogenously added poly(I:C). Further study indicates that poly (I:C) inhibited the differentiation of mouse preadipocytes through PRR-mediated secretion of TNF-α. HSV60 inhibited the differentiation of preadipocytes to mature adipocytes and enhanced the proliferation of adipose cells. Most of these studies have concentrated on immune cells, principally macrophages, dendrite cell and T cells whose metabolic state is also critical to their immune function. The study reports that not only immune cells, but also adipocytes, which are important cells in the body's metabolic state, and their precursor cells during anti-virus infection.

Speaker Biography

Lili Yu focuses her research on the function of pattern recognition receptors in adipose cells and passion *in virus* infection causing weight loss and gain. She got her PhD degree at Peking Union Medical College (PUMC) in Beijing of China in 2014 following the Dr. Daishu Han. Then she worked at Xinxiang Medical University to continue the study which will demonstrate whether these effects observed in vitro can translate to the whole animal and from the mouse model to humans. Currently, she is a visitor at Pennington Biomedical Research Center as a Postdoc.

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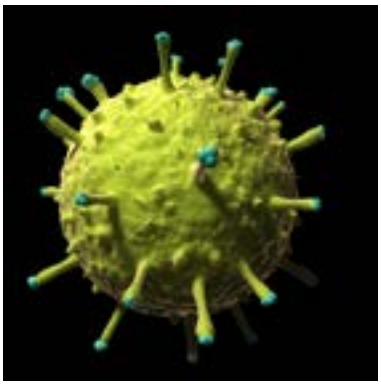

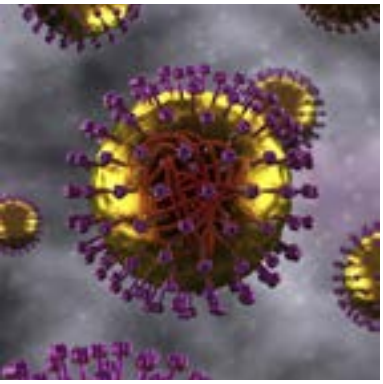

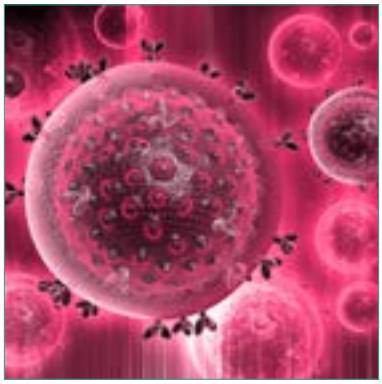

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Roles of cellular DNA replication proteins in papillomavirus DNA replication

Thomas Melendy
University at Buffalo, USA

Other than E1, the papillomavirus (PV) DNA helicase, and E2, the PV transcriptional regulator (that also assists E1 in recognizing the HPV origin of replication and assembling into E1's hexameric helicase configuration), PVs rely entirely on host proteins to replicate their viral DNA. Much of what we know about the enzymes involved in the synthetic stages of eukaryotic DNA replication were initiated in studies using a similar small DNA tumour virus, the polyomavirus, SV40. Studies on SV40 helped identify and/or confirm a role in DNA replication for: DNA polymerase alpha-primase (PolPrim), Topoisomerase I (TopoI), the major cellular ssDNA binding complex (RPA), Replication Factor C, Proliferating Cell Nuclear Antigen, DNA polymerase delta (PolD), and others. Another cellular replication complexes such as DNA polymerase epsilon (PolE), and origin recognition and licensing factors such as: The Origin Recognition Complex, The Mini-Chromosome, Maintenance proteins, Cdc45p, and others, were not required. SV40 hijacks the cellular machinery by its helicase binding to just RPA, TopoI and PolPrim, and the remaining factors are recruited by secondary interactions. A major focus of my laboratory has been on elucidating how the PV DNA replication proteins, E1 and E2, interact with and recruit the cellular replication proteins. We found that as with SV40, the PV helicase, E1 binds to: RPA, TopoI and PolPrim; and we have elucidated some of the mechanisms behind why these interactions are so vital for viral DNA replication. Moreover, we have recently discovered additional interactions unlike those seen in the SV40 system, including interactions between E1 and PolD and PolE and the PV E2 transcription protein with TopoI and PolE. These are highly novel as no other virus has ever been shown to evolve to utilize PolE to replicate their viral genomes, and E1 in particular confers a novel and highly unusual stimulation to synthesis by the cellular PolE enzyme. These findings show that PV DNA replication is actually quite different than polyomavirus DNA replication, from a functional and viral recruitment perspective; and show that PV DNA replication is apparently more similar to cellular DNA replication than polyomavirus (SV40) DNA replication. Further, our results provide novel biochemical targets for development of new anti-PV therapeutics.

Speaker Biography

Thomas Melendy has completed his PhD at UCLA, was a Post-doctoral Fellow with Bruce Stillman (NAS and FRS) at Cold Spring Harbor Laboratory where he wrote and published the seminal Nature article on DNA polymerase switching. He is currently an Associate Professor at the University at Buffalo, where he continues his ground-breaking work on the mechanisms of viral DNA replication. He is an AAAS Fellow, Presidential Scholar, Damon Runyon Fellow, Roche Award winner, served on ACS and NIH Review Panels, has held numerous NIH/ACS grants and career development awards, and has published over 40 papers in highly reputed journals.

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Notes:

Infectivity and host pathogen interaction study of Chickpea chlorotic dwarf virus L strain isolated from cotton

Muhammad Saleem Haider[1], **Muhammad Shafiq, Fakhra Shamim, Muhammad Tariq Manzoor** and **Fasiha Qureshi**
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Cotton leaf curl disease in the Indian subcontinent is associated with several distinct begomoviruses that interact with a disease-specific DNA satellite named Cotton leaf curl Multan betasatellite (CLCuMB). However, we have recently reported the distinct strain (L) of leafhopper transmitted chickpea chlorotic dwarf virus, CpCDV-L (genus Mastrevirus, family Geminiviridae) from cotton plants affected by leaf curl disease in a small number of plants. The question as to whether CpCDV-L contributes to the development of disease symptoms such as leaf curling and enations remain to be answered. Standard methods were used to produce partial direct and tandem repeat constructs of CpCDV-L for *Agrobacterium*-mediated inoculation in the binary vector pBIN19. The role of CpCDV-L in the induction of typical disease symptoms was studied by *Agrobacterium*-mediated inoculation of the partial repeat construct to *Nicotiana benthamiana*. CpCDV-L induced downward leaf curling leading to cup shape in *N. benthamiana*. The complete tandem dimeric construct of the virus was also found to be highly infectious to chickpea, and induced severe stunting of the plant, leaf smalling, drying, and the eventual death of the plant. This strain could be future possible threat to cotton crop in Pakistan.

Speaker Biography

Muhammad Saleem Haider has completed his PhD at the age of 32 years from University of London, Imperial College of Science,Technology and Medicine, London (United Kingdom) and Post Doctorate from the University of Toronto, Canada. He is the director of the Institute of Agricultural Sciences, University of the Punjab, Lahore-Pakistan. He has published more than 100 papers in reputed journals and has been serving as an editorial board member of well reputed journals. Currently, also holding the office of the President, Pakistan Phytopathological Society.

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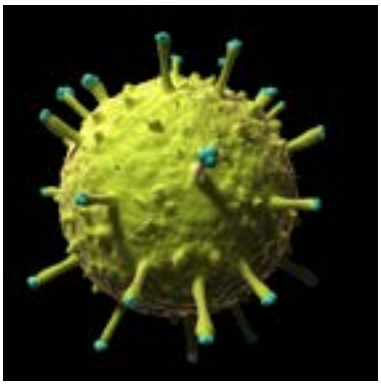

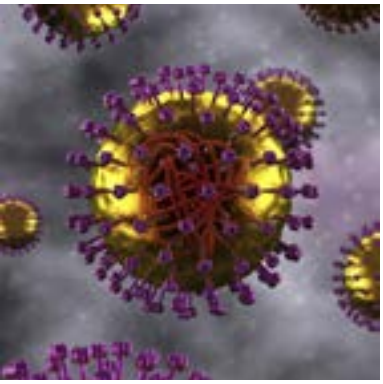

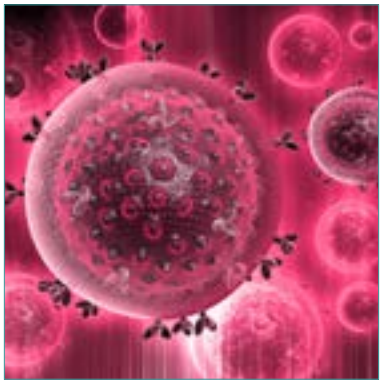

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Coxsackievirus type B3 is a potent oncolytic virus against KRAS-mutant non-small-cell lung cancer

H Deng[1], T D Sliva[2], H Liu[1], Y Xue[1], Y Mohamud[1], J Zhang[1], W Lockwood[2] and **H Luo[1]**
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Background: Lung cancer is one of the most leading causes of cancer-related death worldwide. Over 85% of lung cancers are non-small-cell lung cancer (NSCLC), for which the 5-year survival rate is extremely low (~15.9%). Most NSCLCs are caused by the accumulation of genomic alterations, among which epidermal growth factor receptor (EGFR) mutation and KRAS mutation are two of the most predominant types. Although patients with EGFR-mutant NSCLCs have manifested a good response to EGFR inhibitors, there is a paucity of effective treatments for the KRAS-mutant NSCLCs and new strategies are urgently needed. Coxsackievirus type B3 (CV-B3) is a non-enveloped, human pathogenic enterovirus that causes mild flu-like symptoms in adults. Due to its highly lytic nature, CV-B3 has yielded an increased efficacy of viral-mediated oncolysis as compared to other viruses, which makes it as a good candidate for cancer treatment.

Methods: Seven NSCLC cell lines (A549, H2030, H23, H1975, PC-9, H3255 and HCC4006) and three normal lung epithelial cells (HPL1D, HAE and BEAS2B) were selected for this study. Cells were infected with CV-B3 (MOI 0.01) for 48 hrs. Cytopathic effects caused by virus infection were observed by light microscope, followed by crystal violet staining. MTS assay were conducted to examine the resistance of normal lung epithelial cells upon CVB3 infection. The supernatants were collected to determine the virus titres by plaque assay. Coxsackievirus and adenovirus receptor (CAR) expression was examined via western blot.

Results: Our studies found that CV-B3 treatment led to a significant reduction of cell survival in KRAS-mutant NSCLCs but not EGFR-mutant NSCLCs nor normal lung epithelial cells. MTS assay results demonstrated CV-B3 infection didn't lead to a significant enhancement of cell death in normal lung epithelial cells. Furthermore, we showed that virus titres within the supernatants of KRAS-mutant NSCLCs are significantly higher than both EGFR-mutant NSCLCs and normal lung epithelial cells. Finally, we demonstrated that CAR expression levels were significantly increased in KRAS-mutant NSCLCs.

Conclusions: Our study found that CV-B3 is an effective and safe oncolytic virus against KRAS-mutant NSCLCs.

Speaker Biography

Haoyu Deng is a PhD student from St. Paul's Hospital, Canada. His supervisor is Dr. Honglin Luo and his former major was surgery. The medical science of UBC has become one of its priority fields which are making great contributions to the medical development. His current project is about the functional role of Gab1 in heart disease, especially looking at the mechanisms involved in the role of Gab1 in molecular signaling pathways when cardiomyocytes are infected by CVB3.

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Diversity of geminivirus associated alphasatellites from different cultivated and non-cultivated plants in Pakistan

Fakhra Shamim[1, 2], **Muhammad Shafiq, Misha Jabbar, Tehmina Bahar** and **Muhammad Saleem Haider**

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The viruses belonging to the genus *Begomovirus* are whitefly transmitted monopartite or bipartite viruses. The monopartite *begomovirus*es with their associated satellite-like molecules Alphasatellites are believed to be involved in enhancing the replication of virus and have a major role in breaking host defence. The study presented here involved scanning of different cultivated and non-cultivated plants to detect and isolate new complexes. Samples of leaves were collected from different areas of Punjab, Pakistan and screened for presence of DNA-A, alphasatellite and betasatellite molecules through PCR. A total of 8 alphasatellite, 5 betasatellite and 1 DNA-A full length genome was reported. Phylogenetic studies of alphasatellite molecules were made. 6 sequences isolated from cotton leaves collected from Vihari had close resemblance of up to 90 % similarity with satellite molecules of PaLCuA (*Papaya leaf curl alphasatellite*) and could be new strains of PaLCuA first time reported from cotton plants. The sequence MJ-24[CLCuMA-PK-Multan-cotton] reported from Multan displayed 98% similarity with CLCuMA (*Cotton leaf curl Multan alphasatellite*) indicating that it is a variant of CLCuMA. MJ-25[GDSA-PK-Multan-cotton] also reported from Multan was classified as a new strain of GDSA (*Gossypium dawanii symptomless alphasatellite*) with a score of 93% similarity. Phylogenetic tree further confirmed the results. An analysis of ORF with Rep coding sequence of alphasatellites was made by comparison with earlier reported protein sequences in the database. Results revealed highly conserved regions of Rep domain and helicase binding domain. Only one sequence MJ-25 displayed some substitutions in the Rep domain. Other substitutions were observed in central region or hydroxyl end of protein.

Speaker Biography

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Enterovirus subverts autophagy through cleavage of fusion adaptor proteins and selective autophagy receptors

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Background: Myocarditis is an inflammatory disease of the heart often caused by viral infection, particularly the enteroviruses, such as coxsackievirus B3 (CVB3). Autophagy, an evolutionarily-conserved intracellular degradation pathway, targets misfolded proteins, damaged organelles, and invading pathogens for lysosomal clearance. Although traditionally considered a non-selective degradative process, it's now clear that autophagy can mediate targeted clearance of protein aggregates/damaged organelles via selective autophagy receptors, which harbor highly conserved ubiquitin-associated and LC3 interacting domains. Contrary to previous understanding of autophagy as an anti-viral pathway, we and others have shown that the cellular autophagic machinery can be hijacked by enterovirus to disrupt its degradative capacity (or autophagic flux) and promote the accumulation of autophagosomes that serve as membrane scaffolds for viral replication. Moreover, we discovered that two selective autophagic receptors, namely p62/sequestosome 1 and neighbor of BRCA1 gene 1 (NBR1), are cleaved upon CVB3 infection, resulting in not only loss-of-function, but also the generation of dominant-negative fragments that further impair selective clearance of ubiquitinated protein aggregates. Despite these intriguing findings, the exact mechanism by which CVB3 inhibits autophagic flux and disrupts protein/organelle quality control is not fully understood. We hypothesize that CVB3 infection impairs the autophagic pathway through virus-encoded proteinases that specifically target autophagic proteins required for autophagosome-lysosome fusion and/or selective cargo recruitment, ultimately leading to cardiac dysfunction by facilitating viral replication and via preventing the clearance of toxic protein aggregates/damaged organelles.

Methods & Results: Our previous *in vivo* findings that CVB3-infected mouse hearts display an abnormal accumulation of autophagosomes and misfolded proteins/damaged mitochondria, and the in vitro evidence that CVB3 infection inhibits autophagic flux, suggest that the fusion process of autophagy is disrupted during infection. To delineate the possible mechanism involved, we focused on proteins previously reported to be involved in autophagosome fusion. Notably, we found that the autophagosomal SNARE (soluble N-ethylmaleimide-sensitive factor activating protein receptor) protein SNAP29 (synaptoxomal-associated protein 29) and the tethering protein PLEKHM1 (pleckstrin homology domain containing protein family member 1), two critical proteins known to regulate autophagosome-lysosome fusion, were cleaved upon CVB3 infection. Further *in vivo* (in cells transfected with protease constructs) and in vitro (using recombinant proteases) cleavage assays demonstrated that CVB3-encoded proteinase 3Cpro, not 2Apro or caspases, is responsible for these cleavages. Combining a bioinformatics approach with site-directed mutagenesis, we identified the cleavage sites on SNAP29 (Q161) and PLEKHM1 (Q668), respectively, leading to impaired SNARE complex formation. Moreover, we showed that gene-silencing of SNAP29 and PLEKHM1 inhibited autophagic flux, resulting in a significant increase in viral growth, likely due to enhanced accumulation of autophagosomes that provide sites for viral RNA replication and assembly. Finally, we also identified the autophagic receptor protein, NDP52 (nuclear domain 10 protein 52), as a bona fide substrate of viral proteinase 3Cpro. The cleavage of NDP52 takes place at Q139, separating the N-terminal LC3-interacting region from the C-terminal ubiquitin-binding domain. The functional significance of NDP52 cleavage is currently under investigation.

Conclusion: We identified a novel underlying mechanism by which enterovirus, through viral encoded proteinases, subverts the host autophagic pathway to promote viral propagation and cause cardiac damage. Our findings in this study provide strong evidence of a potential therapeutic benefit by targeting the autophagy-virus interface.

Speaker Biography

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Factors associated with first-line antiretroviral treatment failure in adult patients with HIV; Asella Hospital, Ethiopia: A case-control study

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Background: Treatment failure has become a significant challenge in patients taking Antiretroviral Therapy (ART). The aim of the present study was to identify risk factors for first-line ART failure among patients attending clinical follow-up in Asella Hospital, South Eastern Ethiopia.

Materials/methods: A 1:2 matched case-control study (by age, sex, and duration of ART) was conducted from June 2015 to July 2017 on adult patients (≥ 15 years) who were on ART for at least 6 months. Ninety-one patients who were transferred to second-line ART after confirmed first-line ART failure (viral load ≥ 1000cells/mm3) were cases and 182 patients who did not fail on their first-line ART were controls. Data were collected using interview questionnaire, previous chart records and laboratory tests to detect chronic carrier state for H. pylori, Hepatitis B and C viral infections. Multivariate logistic regression analysis was performed.

Results: From 273 patients who participated in this study; 54.6% were males and 45.4% were females. The average age and duration on ART were 41.4 years and 71.2 months respectively. Independent risk factors associated with ART failure were tuberculosis treatment while on ART (OR=11.08: 95% CI: 4.57-26.87), discontinuation of ART drugs (OR=7.35; 95%CI: 3.92-13.79), persistent or repeated diarrhea (OR=4.64: 95%CI= 1.90-11.31), and advanced baseline WHO Stage IV (OR=4.05; 95%CI: 1.03-16.00). Food made of wheat (OR=1.87: 95%CI: 0.75-4.67), H. pylori co-infection (OR=0.76: 95%CI: 0.41-1.42), Hepatitis B carrier state (OR=0.93: 95%CI: 0.30-2.86), and Hepatitis C carrier states (OR= 0.41: 95%CI: 0.05-3.93) were not significantly associated with antiretroviral treatment failure in this study.

Conclusions: Prevention of tuberculosis and special emphasis on management of HIV and tuberculosis co-infections, counseling patients on adherence to ART drugs and hygiene; and starting ART earlier help to decrease ART failure.

Speaker Biography

Yihienew M. Bezabih has completed his MD (doctor of medicine) degree at age of 27 and graduated with great distinction winning silver medal from Adama University, Ethiopia. He also won the Adama University's high scoring students award in 2010. He currently works as lecturer at Arsi University College of Health Sciences and leads two research projects on HIV and stroke as principal investigator.

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