Mechanisms of virus uncoating

*Ari Helenius
ETH Zurich, Switzerland

When viruses enter, it is not enough that they find their way to the right compartment and location within the host cell. To allow replication and transcription, they also must release the genome, which is condensed and protected within a capsid. By tracking a variety of animal viruses during cell entry and by using in vitro biochemical experiments as well as genome wide loss-of-function screens, my co-workers and I have analysed the uncoating of viruses from several different virus families. In the lecture, I will describe what we have learned about the mechanisms used by alphaviruses, influenza A virus, vaccinia virus, and SV40. These turn out to be remarkably different. However, all of them depend for uncoating on a variety of cues and assistance provided by host cell factors. Uncoating generally occurs in multiple steps, and mechanisms are place to prevent premature capsid disassembly in the producer cell.

Keywords: entry, Capsid, uncoating

*Presenting author

Intra-host evolution of Zika virus during pregnancy

*Teemu Smura, Eili Huhtamo, Essi Korhonen, Suvi Kuivanen, Jussi Hepojoki, Olli Vapalahti
University of Helsinki, Finland

Zika virus (ZIKV), a mosquito-borne flavivirus, has been associated with an increased risk of congenital microcephaly. ZIKV was originally associated with mild febrile disease and maculopapular rash in tropical Africa and some areas of Southeast Asia. Since 2007, ZIKV has caused several outbreaks outside its former distribution area in islands of the Pacific and, since 2015, in South America. A striking feature of the current ZIKV outbreak is the increased risk of intrauterine or perinatal transmission of the virus as well as the marked increase in the number of newborns with microcephaly.

The mutation frequency of RNA viruses is very high, due to the lack of proof-reading activity of viral RNA polymerase. This enables rapid, opportunistic adaptation to changing environments such as replication in different target tissues. During such adaptation event, novel point mutations may enable infection of different target tissues within the host. This process may lead to viral colonization of a new cell type/organ and possibly to changes in pathogenicity.

In order to estimate potential role of viral adaptation during the congenital ZIKV infection, samples collected from a case of a pregnant woman and her fetus infected with ZIKV during the 11th gestational week were subjected to ZIKV-specific PCR and sequencing. Samples were available from the blood of mother during the acute stage of infection as well as the placenta, umbilical cord, fetal brain and the serum of the mother at the time of termination of pregnancy (at 21 weeks of gestation). Next generation sequencing was used to track changes in the viral consensus sequence and to identify the frequencies of minority mutations between virus sequences. The results suggested changes in ZIKV population structure as well as fixation of specific amino acid substitutions during the infection of fetal tissues.

Keywords: flavivirus, Zika virus, evolution

*Presenting author

Zika virus nonstructural protein 5 efficiently interferes with the activation of the RIG-I signaling pathway

*Rickard Lundberg [1], Krister Mélen [2], Miao Jiang [2], Veera Westenius [2], Pamela Österlund [2], Olli Vapalahti [3], Laura Kakkola [1], Ilkka Julkunen [1]
[1]Institute of Biomedicine/Virology, University of Turku, Finland
[2]Viral Infections Unit, National Institute for Health and Welfare, Finland
[3]Department of Virology, University of Helsinki, Finland

Zika virus (ZIKV) is a mosquito-borne virus belonging to the Flaviviridae family. It is an enveloped virus with a 10.8 kb long single-stranded positive-sense RNA genome, which encodes for 4 structural proteins and 7 non-structural proteins. ZIKV was isolated in 1947 from a rhesus monkey living in Ziika forest, Uganda. The following fifty years brought little cause for concern, with only sporadic cases of a very mild disease. This changed in 2007 when ZIKV caused an epidemic in the Yap islands, Micronesia, which resulted in the infection of 80% of the population out of which 20% became symptomatic. Following that outbreak, ZIKV has caused two subsequent epidemics in French Polynesia and the Americas in 2013 and 2015, respectively. During these outbreaks severe complications have been associated with ZIKV infection, such as Guillain- Barré syndrome and microcephaly. This lead to a global health concern, and ZIKV was declared by WHO a public health emergency. Recent studies have shown ZIKV to interfere with human innate immune responses, especially with interferon-induced responses. At present it is not known whether or not ZIKV can also interfere with the RIG-I or TLR signaling cascades and the expression of cytokine genes such as IFN genes.

The present study was initiated to analyze whether any of the ZIKV proteins can interfere with the RIG-I induced IFN-lambda1 gene expression. For this purpose we cloned all 11 ZIKV genes and inserted them into a mammalian expression vector. We co-transfected HEK293 cells with a constitutively active form of RIG-I, IFN-lambda1-luciferase reporter plasmid, and ZIKV expression plasmids. The effect of expressed ZIKV proteins on RIG-I-induced IFN-lambda1-promoter activation was measured with luciferase assay. We found ZIKV-NS2A and especially NS5 proteins to inhibit the RIG-I-mediated activation of IFN-lambda1 promoter. The mechanism of action for these inhibitory effects will be determined in future studies.

Keywords: Flavivirus, Zika Virus, Innate immunity

*Presenting author

Extinct type of human parvovirus B19 persists in tonsillar B cells

Lari Pyöriä#[1], *__Mari Toppinen#__[1], **Elina Mäntylä**[2], **Lea Hedman**[3], **Leena-Maija Aaltonen**[4], **Maija Vihinen-Ranta**[2], **Taru Ilmarinen**[4], **Maria Söderlund-Venermo**[1], **Klaus Hedman**[3], **Maria F Perdomo**[1]
[1]University of Helsinki, Finland
[2]University of Jyväskylä, Finland
[3]University of Helsinki and Helsinki University Hospital, Finland
[4]Helsinki University Hospital, Finland

Parvovirus B19 (B19V) DNA persists lifelong in human tissues, but the cell type harboring it remains unclear. We here explore B19V DNA distribution in B, T and monocyte cell lineages of recently excised tonsillar tissues from 77 individuals with an age range of 2-69 years. We show that B19V DNA is most frequent and abundant among B cells, and within them we find a B19V genotype that vanished from circulation more than 40 years ago. Since re-infection or re-activation are unlikely with this virus type, this finding supports the maintenance of pathogen-specific humoral immune responses as a consequence of B cell -long-term survival rather than continuous replenishment of the memory pool. Moreover, we demonstrate the mechanism of B19V internalization to be antibody dependent in two B-cell lines as well as in ex-vivo isolated tonsillar B cells. This study provides direct evidence for a cell type accountable for B19V DNA tissue persistence.

#These authors contributed equally to this work.

Keywords: antibody dependent enhancement, persistence, B cells, parvovirus

*Presenting author

Differential receptor use of coxsackievirus A9 and human parechovirus 1 – two picornaviruses possessing RGD-motif

*Pirjo Merilahti, Sisko Tauriainen, Petri Susi
University of Turku, Finland

Coxsackievirus A9 (CV-A9) and human parechovirus 1 (HPeV-1) belong to a family of Picornaviridae, more precisely described to Enterovirus and Parechovirus genera, respectively. CV-A9 has been associated with aseptic meningitis, myocarditis as well as other mild or severe infections. HPeV-1 mainly causes mild gastrointestinal and respiratory diseases, but also more severe diseases such as myocarditis and transient paralysis have been observed. CV-A9 and HPeV-1 infections are common in infants and neonates. There are no vaccines, antivirals or drugs against these viruses. CV-A9 and HPeV-1 possess a specific integrin-recognizing arginine-glycine-aspartic acid (RGD) –motif on their capsids, and integrins have therefore been suggested to act as cell surface receptors for these viruses. However, the cellular experiments suggest that CV-A9 is capable to internalize into cells without integrins. In this work we showed that in the absence of RGD-binding integrins, CV-A9 binds to heat shock protein family A member 5 (HSPA5) protein on the cell surface assisted by heparan sulfate (HS) and beta-2-microglobulin (b2M). Our results also suggest that HPeV-1 infection is dependent on RGD-binding integrin alphaVbeta1 as its primary receptor, and that HS and b2M act as accessory receptors for HPeV-1.

Keywords: coxsackievirus A9, human parechovirus 1, receptor

*Presenting author

Viruses and type 1 diabetes – next steps towards prevention trials

*Heikki Hyöty
University of Tampere, Faculty of Medicine and Biosciences, Finland

Several studies have reported enterovirus infections more frequently in type 1 diabetic patients than in control subjects. These viruses have tropism to insulin-producing beta cells, and in line with this, enterovirus protein has been detected in the islets in the majority of diabetic patients. The presence of virus has been confirmed by RT-PCR and in situ hybridization. However, only few cells are usually virus positive in the islets and viral titers are low. These findings fit with a slowly replicating chronic infection in the pancreatic islets, similar to that described in the pancreas and heart in mouse models. Beta cells express receptors which coxsackie B group enteroviruses use to enter the cell. Recent epidemiological studies in large patient cohorts have also supported the role of this enterovirus subgroup in the initiation of beta-cell damaging process. Due to the accumulating evidence linking enteroviruses to the pathogenesis of type 1 diabetes the question of whether a preventive vaccine could be developed to evaluate possible causality of this association. The success of polio vaccines indicates that enterovirus vaccines are generally safe and effective, and technologies to produce such vaccines are in place. The first attempts to develop vaccines against diabetogenic enteroviruses are in progress focusing on group B coxsackieviruses. Prototype Coxsackie B virus vaccines have been tested in mouse models and shown to be effective and able to prevent virus-induced diabetes. The high costs of clinical phase III trial creates a challenge for clinical development, and therefore the concept needs to be scientifically solid. Large international studies are in progress to produce additional information about the strength of the risk association and its documentation in different populations, mechanisms of viral persistence in the islets and the composition of the vaccine. The idea of testing such vaccine in primary prevention trial among young infants with increased genetic risk of type 1 diabetes is attractive from medical, scientific and economical points of view, and the large market opportunity makes this case attractive also for biotech and vaccine companies. In addition to vaccine, antiviral drugs could be used in intervention trials to test if they can improve the function of beta cells in prediabetic and diabetic individuals (cure of persistently infected beta cells). The first such trials are currently in the planning phase.

*Presenting author

Ability of Type 1 Diabetes associated enteroviruses to cause chronic infection in pancreatic cells and use of antiviral drugs to eradicate such infection

*Anni Honkimaa, Amirbabak Sioofy Khojine, Sami Oikarinen, Jutta Laiho, Heikki Hyöty
University of Tampere, Finland

Type 1 Diabetes (T1D) is an immune mediated disease in which insulin producing pancreatic beta-cells are selectively destructed leading to disruption in insulin synthesis and subsequent secretion with clinical manifestations of hyperglycaemia. Genetics has a strong role in T1D; however, genes cannot explain the pathogenesis of the T1D alone, since only a few of the genetically susceptible individuals develop the disease. Enteroviruses have been linked to T1D in many studies and the most plausible mechanism seems to be a chronic infection in pancreatic beta cells being responsible at least partially for the disease. Most commonly Coxsackie B group viruses have been linked to the disease, and Coxsackievirus B1 (CVB1) seems to be associated with the increased risk of T1D.

We have established a chronic infection models, by four strains of CVB1, in pancreatic epithelial cell line named PANC-1 and by three stains of CVB1 in a beta cell line named 1.1B4. The virus have been replicating for 18 months in serial passages of two chronic infection models achieved by ATCC and a clinical isolate of CVB1. The presence of infectious virus have been verified by different techniques including RT-PCR, virus plaque titration assay, immunofluorescent staining, immunohistochemistry, and in situ hybridization. The effect of antiviral drugs was identified against representative members of CVB group to show the serotype specificity of the drug. The chronic infection model in pancreatic cell line was then employed to identify the effect of antiviral strategies. Pleconaril, a well known pocket factor replacing agent of Picornaviruses, was shown to be serotype and strain specific. We showed that it prevents the ATCC strain of CVB1 cytopathic effect in A549 cells in an acute infection model. The drug was also effective against the same strain in our chronic infection model in PANC-1 cells, with 1 uM concentration.

Keywords: Chronic infection, Type 1 Diabetes, Enterovirus, Antiviral

*Presenting author

High throughput screening in search for novel regulators and antivirals of enterovirus infection

*Mira Myllynen[1], Mari Martikainen[1], Anni Honkimaa[2], Juha Määttä[2], Famara Sane[3], Evgeny Kulesskiy[4], Jerolen Naidoo[5], Peter Horvath[6], Reka Hollandi[6], Vesa Hytönen[2], Heikki Hyöty[2], Denis Kainov[4], Didier Hober[3], Musa Mhlanga[5], Varpu Marjomäki[1]
[1]Department of Biological and Environmental Science/Nanosciene center, University of Jyväskylä, Jyväskylä, Finland
[2]Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland
[3]Laboratory of Virology EA3610, University Lille 2, CHRU Lille, Lille, France
[4]Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland
[5]The Council for Scientific and Industrial Research (CSIR), Pretoria, South Africa
[6]Synthetic and Systems Biology Unit, Hungarian Academy of Sciences, Biological Research Center (BRC), Szeged, Hungary

Enteroviruses belong to a large family of non-enveloped viruses, picornaviridae. The enterovirus B subgroup consists of the clinically important human pathogens: echovirus 1 (EV1), and coxsackieviruses A9 (CAV9), and B1 (CVB1) – B6 (CVB6). These viruses cause severe outbreaks especially among children, with symptoms varying between mild infections and severe states such as aseptic meningitis, heart muscle damage and paralysis. At the moment, the information on crucial cytoplasmic cellular factors regulating infection is largely lacking but it would be essential in understanding the mechanisms of acute and chronic infections and in developing drugs against enterovirus infections. Here, we have used two high throughput screening approaches to reveal new cellular regulators of enterovirus infection. We carried out a drug screen targeting cellular regulators as well as a large microRNA screen, which both revealed several potential hits that prevented enterovirus infection. Further studies on one of the drug screen hits showed that the drug blocked the infection of EV1, CVA9 and CVB1-CVB6 in vitro. Furthermore, the efficacy was proven in vivo in mice where levels of infectious CVB4 were significantly decreased in pancreas and heart tissue. The effect of the drug was also evident at the messenger RNA level, when the drug attenuated viral induced effects in a transcriptomics study. Our preliminary mechanistic studies showed that the drug has a direct effect on the virus nonstructural proteins in addition to the known cellular target. The drug was shown to prevent viral replication and capsid production and to have a special inhibitory effect on the viral 3C protease. By learning the mechanistic basis for the different viral inhibitors not only increases our understanding of the enterovirus infection, but also opens new avenues for ways to inhibit viral infection for a large group of human pathogens.

Keywords: inhibitor, high throughput screening, enterovirus

*Presenting author

Topical treatment of herpes simplex virus infection with an enzymatically created Dicer-substrate siRNA swarm in a keratitis model

*Henrik Paavilainen[1], Jenni Lehtinen[1], Alesia Romanovskaya[2], Michaela Nygårdas[1], Dennis H Bamford[2], Minna M Poranen[2], Veijo Hukkanen[1]
[1]University of Turku, Finland
[2]University of Helsinki, Finland

Herpes simplex virus (HSV) is a common human pathogen. Of clinical symptoms, the most common one is a blister on the lip. HSV infection can manifest at other sites of the body as well. Despite current antivirals, HSV infections of the eye are the leading cause of blindness due to infectious origin. Drug resistant strains exist and they are especially prevalent in immunocompromised patients and in HSV eye infections. New treatment modalities are needed. Previously, we have shown that our antiviral siRNA swarms are effective against HSV infection of both laboratory and clinical isolates of HSV in vitro and that innate immunity has a small role in HSV inhibition by these swarms. In this study, we investigated the antiviral effect in vivo in an HSV keratitis model.

BALB/c mice were corneally infected with HSV and subsequently treated with a swarm of enzymatically created, Dicer-substrate small interfering RNA (siRNA) molecules targeting HSV gene UL29. Unspecific siRNA swarm and buffer only were used as control treatments. Two infection models were used: a peripherally oriented and CNS spreading infection. Mouse survival, as well as viral spread, load, latency, and peripheral shedding, were studied.

The anti-HSV-UL29 siRNA swarm inhibited HSV. The swarm alleviated HSV infection symptoms ($p<0.01$), had a favorable effect on mouse survival ($p=0.063$) and decreased viral load in tissues ($p<0.05$). Viral shedding and replication were also inhibited at the peak of peripheral infection ($p<0.05$).

We show here, that an antiviral treatment with siRNA swarms can be effective against HSV keratitis in a mouse model. Our enzymatically created Dicer-substrate anti-HSV-siRNA swarm was able to inhibit HSV replication by one log and protect the mice from viral CNS spread with one dose administration. Our results demonstrate that a topical RNA interference approach against HSV keratitis is feasible.

Keywords: RNA interference, Herpes simplex virus, Antiviral, siRNA

*Presenting author

Disruption of methionine cycle- a potential strategy undertaken by potyviral HCPro to suppress anti-viral RNA silencing in the host during single and mixed infection scenario

*Swarnalok De, Konstantin Ivanov, Marta Basic, Andres Lõhmus, Kristiina Mäkinen
University of Helsinki, Finland

Methionine cycle is a biochemical pathway widely operating in both animal and plant systems. It produces ubiquitous methyl-group donor S-adenosyl-methionine (SAM), which is subsequently used by many methyltransferases to methylate a broad range of biomolecules. Methionine cycle plays an important role in smooth running of antiviral RNA silencing pathways in the hosts. During viral infection, dicer processed viral dsRNA intermediates (sRNAs of typically 21-24 nucleotides) are protected from degradation through 2´–O-methylation of their 3´ ends by the SAM-dependent methyltransferase HEN1. In this study, HCPro, the anti-viral RNA silencing suppresor of potato virus A (PVA; genus potyvirus) has been shown to interact with SAM synthetase (SAMS) and SAH hydrolase (SAHH). HCPro-mediated inhibition of SAMS is hypothesized to deprive HEN1 of its substrate SAM. As a result, HEN1 is unable to methylate sRNAs, which leads to the disruption of the antiviral RNA silencing pathway through 3´ polyuridylation and degradation of unmethylated sRNAs. The effect is thought to be enhanced further by the inhibition of SAHH, causing accumulation of the HEN1 inhibitor SAH. Partial rescue of HCPro less PVA by silencing SAMS and SAHH reinforced the idea.

The study also shows HCPro mediated-disruption of methionine cycle could be one of the factors underlying synergistic interaction between potato virus X (PVX, genus potexvirus) and PVA. Among the components of methionine cycle, SAHH was found to have a role in the synergistic reaction between PVA and PVX. Similarly to co-expression of PVA HCPro with PVX, PVX infection in SAHH silenced background consistently demonstrated enhancement in PVX titre. It has been reported that PVX (-)-strand synthesis is specifically upregulated during poty-potexviral co-infection. We observed a 2-fold increase in the (-)-strand synthesis of PVX-RNA when HEN1 and SAHH were knocked down also reinforcing the role of methionine cycle in the mechanism of poty-potexvirus synergistic interaction.

Keywords: Methionine cycle, PVX-PVA synergism, silencing suppression

*Presenting author

Analysis of potential immunological cross-reactivity with hypocretin receptors and influenza A virus nucleoprotein in Pandemrix vaccine-related narcolepsy

Krister Melen [1], **Jyrki Kukkonen** [2], **Markku Partinen** [3], **Outi Vaarala** [2], **Arja Vuorela** [2], **Seppo Meri** [2], **Hanna Nohynek** [4], *__Ilkka Julkunen__ [5]
[1]University of Turku/THL, Finland
[2]University of Helsinki, Finland
[3]Helsinki Sleep Clinic, Finland
[4]National Institute for Health and Welfare, THL, Finland
[5]University of Turku, Finland

After 2009 influenza A virus pandemic a number of children and adolescent developed narcolepsy. The incidence of narcolepsy among children and adolescent was increased 3-16 fold over the baseline level is many European countries, where Pandemrix vaccine was used. There was strong evidence that the onset of narcolepsy was associated with the use of Pandemrix vaccine. In order to understand the mechanisms of Pandemrix-associated narcolepsy blood cell and serum specimens were collected from narcoleptic patients in 2010-2012. Initial analyses revealed that practically all patients were positive for the narcolepsy risk genotype HLA class II DQB1:0602 and many patients had antibodies against neuronal structures as analysed by immunohistochemistry using rat brain sections. In addition, in a limited analysis, some patients were observed to have antibodies against a cross-reactive epitope between hypocretin receptor 2 and influenza A virus nucleoprotein. In the present study we carried out a more systematic analysis of antibody responses against potential cross-reactive epitopes between human hypocretin receptors and influenza A virus nucleoprotein using serum specimens collected from narcoleptic patients (n=56), adults vaccinated with Pandemrix (n=20) and patients who suffered from a PRC-confirmed influenza A virus infection (n=28). Hypocretin receptors were successfully expressed by baculovirus expression and transfection of receptor gene constructs in human HuH7 hepatoma cell line. In addition, the potential cross-reactive epitopes and corresponding mutant forms of hypocretin 2 and influenza A virus nuclepotein were expressed as peptides and GST-fusion proteins. We found efficient immune response against influenza A virus proteins among all study groups, but failed to show convincing evidence that the narcoleptic patients have cross-reactive humoral immune responses against a common epitope between hypocretin receptor 2 and influenza A virus NP.

Keywords: vaccination, narcolepsy, hypocretin, influenza

*Presenting author

Modifications of JC and BK polyomavirus noncoding control regions in the pathogenesis of severe chronic diseases

*Hanna Seppälä[1], Elina Virtanen[1], Pia Laine[2], Lars Paulín[2], Petri Auvinen[2], Eeva Auvinen[1]
[1]Department of Virology, Medicum, University of Helsinki and HUSLAB, Helsinki, Finland
[2]DNA Sequencing and Genomics Laboratory, Institute of Biotechnology, University of Helsinki, Helsinki, Finland

Long-lasting immunosuppression may reactivate latent human polyomaviruses and cause rare, yet serious, chronic diseases. Lytic infection of brain glial cells by reactivated JC polyomavirus (JCPyV) may cause progressive multifocal leukoencephalopathy (PML) in HIV patients and patients treated with biological immunomodulatory drugs. Reactivation of BK polyomavirus (BKPyV) in the kidneys may result in polyomavirus-associated nephropathy (PyVAN) after kidney transplantation. Both viruses can be characterized based on the architecture of viral noncoding control region (NCCR). Mutated strains develop from circulating archetype strains via rearrangements, deletions and duplications within the NCCR. PML pathogenesis is associated with the development of mutated, neurotropic JCPyV strains that always have modifications in the NCCR. Mutated BKPyV strains have also been found in kidney transplant patients but their role in the pathogenesis of PyVAN is unknown.

Next-generation sequencing (NGS) enables extremely detailed description of complex viral populations. Using Illumina Miseq we have been able to sequence complete NCCR regions in one read and in such a depth that even very small archetype or neurotropic viral populations can be characterized. In our recent study, we showed dominance of multiple neurotropic JCPyV strains in the cerebrospinal fluid of a PML patient. We then wanted to characterize BKPyV NCCR regions in plasma samples of kidney transplant patients with suspected PyVAN.

Our preliminary results suggest that archetype BKPyV NCCR is dominant in all plasma samples indicating the essential role of archetype strains in the pathogenesis of PyVAN. In addition, mutated NCCR regions were identified from a majority of samples. Further studies are needed to evaluate the role of mutated strains in the pathogenesis of JCPyV- and BKPyV-associated diseases. To shed more light on this matter, we are currently characterizing JCPyV strains of a kidney transplant patient diagnosed with extremely rare JCPyV-associated nephropathy.

Keywords: NGS, JCPyV, BKPyV, NCCR

*Presenting author

Rapid testing for high-risk HPV in cervical and tonsillar paraffin-embedded tissue using a cartridge-based assay

*Elina Virtanen, Pekka Laurila, Jaana Hagström, Pekka Nieminen, Eeva Auvinen
University of Helsinki and Helsinki University Hospital, Finland

Introduction: High-risk human papillomavirus (hrHPV), the established cause of cervical cancer, is also associated with head and neck carcinomas. These carcinomas have two distinct, histologically indistinguishable subsets of disease: HPV-positive and HPV-negative cancers, and the cancers caused by HPV infection respond considerably better to treatment and have altogether better prognosis.

Materials and methods: In this study, the suitability of a cartridge-based Xpert HPV test (Cepheid, Sunnyvale, CA) for cervical and tonsillar formalin-fixed paraffin-embedded (FFPE) tissue samples was evaluated. Cervical biopsies and liquid cytology samples were collected from 48 women attending colposcopy. Liquid cytology samples were tested using Xpert and Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD) hrHPV tests, and biopsies were processed for histology and tested for hrHPV using Xpert HPV. Further, 29 archived tonsillar carcinoma samples were tested using Xpert, and the results were compared to histology and immunohistochemical p16INK4a (p16) staining.

Results: Among valid cervical liquid cytology samples 46.8% were hrHPV positive using the Xpert test and 55.3% with HC2. The sensitivity of Xpert was 84.6% as compared to HC2, and overall test concordance was 91.5%. Test concordance between valid Xpert results from biopsies and liquid cytology samples was 84.6%. Among valid tonsillar samples 70.4% were hrHPV positive, and a concordance of 96.3% was found between Xpert and p16 staining.

Conclusions: Xpert HPV test cartridge provides a rapid platform to test individual samples, including FFPE samples, which are routinely available from cancer patients. Further studies are needed to establish whether test sensitivity is sufficient to reliably differentiate between hrHPV-positive and hrHPV-negative head and neck carcinomas.

Keywords: carcinoma, Xpert, FFPE, HPV

*Presenting author

New mariPOC influenza A virus test with significantly improved sensitivity

*Petri Antikainen[1], Päivi Suomala[2], Sergey Klotchenko[3], Andrey Vasin[3], Janne Koskinen[1]
[1]ArcDia International Ltd, Finland
[2]Eastern Finland Laboratory Centre Joint Authority Enterprise (ISLAB), Finland
[3]Research Institute of Influenza, Russia

The influenza A virus (IAV) method of the multianalyte Respi test in the mariPOC test system (ArcDia International Ltd, Turku, Finland) has shown 71–92% sensitivity and over 99.5% specificity compared to PCR (Ivaska et al. 2013; Sanbonmatsu-Gámez et al. 2015). To narrow the sensitivity gap between PCR and mariPOC, a new IAV test with higher sensitivity was developed using next generation bioaffinity reagents targeting a conserved epitope within the viral nucleoprotein. The new test shows one order of magnitude better analytical sensitivity against the seasonal IAV subtypes (H1N1, H1N1 swine and H3N2) than the old test. In addition, the new test recognises broadly potentially pandemic IAV subtypes (H2N2, H5N1, H7N3, H7N9 and H9N2). In this study, the clinical performance of the new IAV test was compared to the old IAV test with nasopharyngeal aspirates.

Nasopharyngeal aspirates (N=198), leftover samples from routine diagnostics, were collected in ISLAB laboratory (Mikkeli, Finland) during the influenza season of 2015–2016. The samples were analysed retrospectively according to manufacturer's instructions with the old and new mariPOC IAV tests. Samples with discrepant results were resolved using PCR and/or DFA.

The new mariPOC IAV test was true positive for 13 samples. The old test detected 10 true positives. Thus, the new test found 30% more positive samples compared to the old test. Specificities for the new and the old IAV tests were 100% (185/185) and 98.9% (183/185), respectively.

Our results indicate that the new mariPOC IAV test enables the detection of seasonal as well as potentially pandemic IAV subtypes with significantly improved analytical and clinical sensitivity. The new test improves diagnostic accuracy and clinical management of influenza patients.

Keywords: influenza A virus, point-of-care testing, mariPOC

*Presenting author

Development and characterization of pan-parechovirus antibodies with diagnostic potential

Lav Tripathi[1], **Neus Sanchez-Alberola**[2], **Laura Teixido**[2], **Pirjo Merilahti**[1], **Sisko Tauriainen**[1], **Eeva-Christine Brockmann**[1], **Urpo Lamminmäki**[1], *__Petri Susi__[1]
[1]University of Turku, Finland
[2]AntibodyBcn, Spain

Human parechoviruses (HPeV) are common picornaviral pathogens in children with high seroprevalence before the age of five. Currently, nineteen (19) parechovirus types are known. Parechoviruses have been linked to gastroenteritis and respiratory infections, and more recently to severe CNS disease especially in infants. Currently there are no other means for the detection of HPeVs except RT-qPCR. The aim of this study was to develop antibodies for the detection of human parechoviruses. First, we used purified HPeV-1 (Harris strain) as an immunogen and found that rabbit anti-HPeV-1 antiserum detected HPeV types from 1 to 6. This was suggestive of conserved site(s) within HPeV types. Sequence analysis of capsid protein-encoding genes suggested a conserved motif in VP0 protein. We then generated recombinant VP0 protein in E. coli (HPeV-1-VP0) and used it to immunize rabbits; polyclonal HPeV-1-VP0 also detected HPeV types. HPeV-1 (virus) and HPeV-1-VP0 (protein) were then used as immunogens to obtain mouse hybridoma cell lines. One thousand (1000) cell clones were screened and a single pan-parechovirus mono-specific antibody was identified against VP0. The same antigens were used in biopanning approach (against human phage display scFv antibody library), and one HPeV-1-specific and two pan-HPeV scFv antibody clones were identified. The specificities of the antibodies were tested on ELISA and IFA (DFA). We will describe our experiences in obtaining pan-parechovirus antibodies using different platforms and discuss their diagnostic potential.

Keywords: scFv, parechovirus, hybridoma, polyclonal antiserum

*Presenting author

REPTARENAVIRUS CO-INFECTION IN SNAKES WITH BOID INCLUSION BODY DISEASE (BIBD) AND VERTICAL TRANSMISSION

Udo Hetzel[1], **Saskia Keller**[2], **Eva Dervas**[2], **Tarja Sironen**[3], **Yegor Korzyukov**[3], **Olli Vapalahti**[4], **Anja Kipar**[1], *__Jussi Hepojoki__[5]
[1]University of Zurich / University of Helsinki, Switzerland
[2]Univesity of Zurich, Switzerland
[3]University of Helsinki, Finland
[4]University of Helsinki / Helsinki University Central Hospital Laboratory Diagnostics, Finland
[5]University of Helsinki / University of Zurich, Finland

BIBD is an infectious disease threatening captive boid snakes worldwide. The causative agent of BIBD remained unknown until 2012, when we and others identified novel arenaviruses in snakes with BIBD. The discovery led to establishment of a novel genus, Reptarenavirus, within the family Arenaviridae. Soon after, we and others observed that snakes with BIBD most often carry multiple reptarenavirus L and S segments (arenaviruses are bisegmented). While chronically affected animals often die with secondary bacterial and protozoal infections, Boa constrictor snakes with BIBD can appear clinically healthy. This raised the question, whether parental reptarenavirus infection are passed on to the offspring. To demonstrate vertical transmission of reptarenaviruses, we studied a few Boa constrictor clutches with BIBD-positive parental animals. The samples included the parents, their embryos/foetuses, neonates/perinatal abortions and juveniles. We utilized histology, immunohistochemistry and transmission electron microscopy to confirm BIBD, and next-generation sequencing (NGS) in combination with virus-species specific RT-PCR to confirm reptarenarenavirus infection. Additionally, we established primary tissue cultures of foetal organs. We were able to provide strong evidence of direct reptarenavirus transmission from both parental (maternal and paternal) animals to the offspring. Furthermore, we observed that co-infecting reptarenaviruses are vertically transmitted. However, not all offspring of each clutch obtained the same virome. While embryos/foetuses and perinatal abortions did not consistently show the hallmark of BIBD i.e. intracytoplasmic inclusion bodies in cells, we could show that juveniles from the age of 2 months onwards showed progressive development of BIBD. This indicates that reptarenavirus infection likely leads to the development of the characteristic cytoplasmic viral inclusion bodies (and thereby the disease) over time. Histological findings further suggest that the vertical virus transmission is mediated by oocytes/spermatozoa and/or by the placental chorioallantois. The results rise questions about a possible pathogen-host adaptation, persistent reptarenavirus infection, within the captive boid populations.

Keywords: co-infection, next-generation sequencing, reptarenavirus, vertical transmission

*Presenting author

Archeovirology

*__Klaus Hedman__
University of Helsinki, and Helsinki University Hospital, Finland

The study of viruses in ancient human remains thus far has been limited by low copy numbers, nucleic acid instability and other challenges inherent to ancient DNA. These issues now can be overcome through targeted capture coupled to deep sequencing, and expert data analysis. We lately searched for human DNA viruses, skeletal remains of Finnish WWII casualties unearthed from Karelian wilderness. We found in the remains highly interesting viral sequences, such as (in exclusive occurrence) an extinct B19 virus type, showing that archival bones hold the key to virus history and epidemiology. We then examined soft tissue samples (skin; gut) of 10 mummified remains from a crypt in Vilnius, Lithuania, and obtained comprehensive sequence info of heavily fragmented (noninfectious) variola major genomes. The latters' phylogenetic analysis disclosed for the smallpox virus an evolution more recent than thought, with this basal form dating back to not beyond 1580. These recent reports, along with the few others that have appeared on archeovirology, highlight the vast potential of the emerging approaches enabling new insights into viral origins, adaptation and pathogenicity.

Keywords: smallpox, mummy, evolution, sequencing

*Presenting author

Ancient origins, dispersal and diversity of HPV16, the most oncogenic human papillomavirus

*Ville Pimenoff [1], Sara Nicolas-Parraga [1], Cristina Mendes de Oliveira [2], Ignacio Bravo [3]
[1]Catalan Institute of Oncology – IDIBELL, Spain
[2]Virology Laboratory, Institute of Tropical Medicine, University of Sao Paulo, Brazil
[3]National Center for Scientific Research (CNRS), France

Every human suffers through life a number of papillomaviruses (PVs) infections, most of them asymptomatic. A notable exception are persistent infections by Human papillomavirus 16 (HPV16), responsible for most infection-driven anogenital cancers. Genetic variation within HPV16 exhibits some geographic structure, and oncogenic potential is not homogeneous among HPV16 lineages. Here, we present the most comprehensive study of the evolution and diversity of HPV16, the most oncogenic infectious agent for humans.

We have characterised the global HPV16 viral diversity in the largest series of cancers of the cervix, vulva, vagina, anus and penis. We have further analysed the most comprehensive phylogeographic sample of HPV16 variant complete genomes and of global variant isolates so far reported. We have finally compared the geographic distribution of human and of HPV16 genetic diversity and explored different alternative scenarios for HPV16 origin, evolution and dispersal.

Differential prevalence of HPV16 variants in anogenital cancers exhibited less than 3% of the variance by anatomy, and only one third of the total HPV16 diversity distribution worldwide could be explained by genetic dispersals of modern humans.

More importantly and in contrast to the current evolutionary prediction that HPVs have coevolved exclusively with modern humans, our Bayesian MCMC inference for HPV16 genomes significantly supported a model of viral transmission between archaic and ancestral modern humans. Furthermore, MCMC inference showed that irrespective of the evolutionary rate prior, divergence time for HPV16 lineages predated the recent out-of-Africa migration of modern humans 60–120 kya.

Altogether, we propose that the repertoire of the HVP16 variants coevolved with archaic humans, the diversity of keratinocyte differentiation and innate immune genes that have undergone adaptive introgression, and eventually the host-switch interaction between viral and host genotypes may be largely responsible for the differential association of certain HPV16 variants with cancer risk in certain modern human populations.

Keywords: human papillomavirus, sexually transmitted infection, infection and cancer, virus-host coevolution

*Presenting author

Seroepidemiology of novel human parvoviruses

*Elina Väisänen[1], Ushanandini Mohanraj[1], Paula M. Kinnunen[2], Pikka Jokelainen[3], Mohammadreza Sadeghi[4], Ali M. Barakat[5], Olli Vapalahti[6], Haider Al-Hello[7], Klaus Hedman[6], Maria Söderlund-Venermo[1]
[1]University of Helsinki, Finland
[2]University of Helsinki, Finland; Finnish Food Safety Authority Evira (current), Finland
[3]University of Helsinki, Finland; Statens Serum Institut, Denmark; Estonian University of Life Sciences, Estonia, Finland
[4]Blood Systems Research Institute, CA, USA; University of California San Francisco, CA, USA
[5]Al-Hussein Teaching Hospital, Iraq
[6]University of Helsinki, Finland; Helsinki University Hospital, Finland
[7]National Institute for Health and Welfare (THL), Finland

The three protoparvoviruses, bufavirus (BuV), tusavirus (TuV) and cutavirus (CuV) are the newest human parvoviruses described: all were originally discovered in feces of diarrheic children – BuV in 2012 in Burkina Faso, TuV in 2014 in Tunisia and CuV in 2016 in Brazil and Botswana. To date, three genotypes of BuV have been identified, and the BuV DNA has been detected in one nasal swab and at a low prevalence of 0.3% to 4% in feces of diarrhea patients in Africa, Europe and Asia. TuV DNA has been found only in the original article. CuV has, besides in feces, been detected in the cancerous skin tissue of four French patients with cutaneous T-cell lymphoma and in one Danish patient with melanoma. However, the etiological role of these viruses in human disease remains uncertain.

In our previous study, we found 3.1% of 228 children and 5.6% of 180 healthy adults in Finland to be BuV-IgG positive, whereas only one (0.4%) child had TuV IgG. Interestingly, although 91% of all adults were of Finnish descent, half of the BuV IgG-positive subjects originated from Asia. To study further the geographical differences and the possible contribution of human-animal contact, we included the newly discovered CuV to our IgG EIA panel, and found that among Finnish veterinarians (n=324) 2.8% had BuV and 5.3% CuV IgG antibodies. In striking contrast, >80% of Iraqi healthy adults (n=100) were BuV seropositive while CuV IgG was rare.

These are the first studies describing the presence of protoparvovirus antibodies in humans. In Finland, the seroprevalences of all three viruses were low; nevertheless, BuV infections were found to be very common in the Middle East. Contrary to BuV, CuV infections seem to be more prevalent among Finnish veterinarians. TuV antibody findings were scarce, and should be interpreted with caution.

Keywords: serology, IgG, Protoparvovirus

*Presenting author

DUAL USE RESEARCH – FROM BENCH TO PUBLICATION

*Liina Voutilainen[1], Anna Katz[1], Susanna Sissonen[2], Klaus Hedman[3], Antti Sajantila[4], Simo Nikkari[1]
[1]Centre for Biothreat Preparedness/Finnish Defence Forces, Finland
[2]Centre for Biothreat Preparedness/National Institute for Health and Welfare, Finland
[3]Department of Virology, University of Helsinki/Helsinki University Hospital, Finland
[4]Department of Forensic Medicine, University of Helsinki, Finland

The possibility that research might result in misuse, either intentionally or accidentally, is a long-standing concern in science. "Dual use research" is life science research that is intended for benefit, but which might easily be misapplied to do harm.

Information from virus research can be misapplied to create biological weapons, to bypass or diminish the effectiveness of medical countermeasures, or to threaten in other ways the health and safety of humans, animals, plants, and the environment.

Individuals involved at any stage of life science research have an ethical obligation to avoid or minimize the risks and harm that could result from malevolent use of research outcomes.

Practical guidelines are needed for research with dual use potential:

- how to recognize dual use potential?

- what is my responsibility as a researcher, PI or reviewer?

- how laws and recommendations regulate dual use research?

- how to assess risks in microbiological research?

- which authorities to inform or consult?

- how should dual use research be published?

As an example, we use a recent study with a potential of dual use, where fragments of variola (smallpox) virus were discovered in a 17th century mummy and finally, the whole genome of the ancient virus strain was reconstructed in silico and published.

In conclusion, a proper risk assessment for any microbiological research should be performed. To support this, practical guidelines for effective implementation should be generated.

Keywords: biosecurity, dual use research, biosafety, smallpox

*Presenting author

The RNA takes it all: assembly in human parechovirus

*Sarah Butcher
University of Helsinki, Finland

Assembly of the major viral pathogens of the Picornaviridae family is poorly understood. Human parechovirus 1 is an example of such viruses that contains 60 short regions of ordered RNA density making identical contacts with the protein shell. We used a combination of structural analysis, RNA-based systematic evolution of ligands by exponential enrichment, bioinformatics analysis and reverse genetics to show that these RNA segments are bound to the coat proteins in a sequence-specific manner. Disruption of either the RNA coat protein recognition motif or its contact amino acid residues is deleterious for viral assembly. The data are consistent with RNA packaging signals playing essential roles in virion assembly. Their binding sites on the coat proteins are evolutionarily conserved across the Parechovirus genus, suggesting that they represent potential broad-spectrum anti-viral targets.

References

Shakeel et al. Nature Communications 2016 DOI: 10.1038/ncomms11387

Shakeel et al. Nature Communications 2017 DOI: 10.1038/s41467-016-0011-z

Keywords: SELEX, assembly, parechovirus, packaging

*Presenting author

Non-equilibrium molecular dynamics simulations of the binding affinities of natural lipids, drug-like molecules and gold nanoclusters to hydrophobic pockets of Echovirus 1

*Emmi Pohjolainen, Gerrit Groenhof, Hannu Häkkinen
University of Jyväskylä, Finland

Bio-compatible gold nanoclusters have recently been utilized as site-specific contrast agents for virus imaging in electron microscopy.(1,2) Two strategies have been shown to be successful, either via covalent binding to cysteine groups in the viral capsid (1) or via non-covalent binding to hydrophobic pockets. (2) The latter strategy is expected to prove useful for dynamic studies of virus uncoating, due to the speculated important role of the hydrophobic pockets for virus (in)stability.

We have estimated the binding affinities of the natural pocket factor (palmitic acid), a Pleconaril molecure (antiviral drug) and an in-house synthesized drug-like molecule (Kirtan) into the hydrophobic pocket of Echovirus 1 (EV1) by using molecular dynamics simulations combined with non-equilibrium free energy calculations. (3) We have also studied the binding of Au102pMBA44 nanocluster / Kirtan complex to the pocket, in different protonation states of the cluster surface. Although the absolute binding affinities are overestimated for all the systems, the trend is in agreement with recent experiments. (2) These results suggest that the natural pocket factor can be replaced by molecules Pleconaril or Kirtan1 that have higher estimated binding affinities. Including the gold nanocluster does not decrease the affinity of Kirtan molecule to the virus, but the affinity could be sensitive to the protonation state of the nanocluster, i.e., to pH conditions.

(1) V. Marjomäki, T. Lahtinen, M. Martikainen, J. Koivisto, S. Malola, K. Salorinne, M. Pettersson and H. Häkkinen, "Site-specific targeting of enterovirus capsid by functionalized monodisperse gold nanoclusters", Proc. Natl. Acad. Sci. USA 111, 1277 (2014).

(2) M. Martikainen, K. Salorinne, T. Lahtinen, S. Malola, P. Permi, H. Häkkinen and V. Marjomäki, "Hydrophobic pocket targeting probe for enteroviruses", Nanoscale 7, 17457 (2015).

(3) E. Pohjolanen et al, manuscript in preparation (2017).

Keywords: hydrophobic pocket, gold, molecular dynamics simulation, echovirus

*Presenting author

Coat protein regulation by CK2, CPIP, HSP70, and CHIP is required for potato virus A replication and coat protein accumulation

*Andres Lõhmus [1], Anders Hafrén [2], Kristiina Mäkinen [1]
[1]University of Helsinki, Finland
[2]Swedish University of Agricultural Sciences, Sweden

Potato virus A (PVA; Genus Potyvirus) exploits a polyprotein expression strategy for coat protein (CP) production. Without additional means of regulation this strategy results in CP being produced simultaneously and in an equimolar ratio with the replication proteins. From our previous studies we know that an excess of CP fully blocks viral RNA translation and phosphorylation reduces the RNA binding capacity of PVA CP. Thus, the production of CP and its interaction with viral RNA has to be regulated, to allow for optimal virus multiplication. How the regulation of CP levels during potyvirus infection is achieved is not yet fully understood. We demonstrate here that both CP phosphorylation by protein kinase CK2 and a chaperone system formed by two heat shock proteins, CP-interacting protein (CPIP) and heat shock protein 70 (HSP70), are essential for PVA replication and that all these host proteins have the capacity to contribute to the level of PVA CP accumulation. An E3 ubiquitin ligase called carboxyl terminus Hsc70-interacting protein (CHIP), which may participate in the CPIP-HSP70-mediated CP degradation, is also needed for robust PVA gene expression. Residue Thr243 within the CK2 consensus sequence of PVA CP was found to be essential for viral replication and to regulate CP protein stability. We found that phosphorylation deficient mutant and CK2 silencing inhibited, whereas phospho-mimetic mutant and overexpression of CK2 increased, PVA translation. Together, these findings suggest that binding by non-phosphorylated PVA CP represses viral RNA translation, involving further CP phosphorylation and CPIP-HSP70 chaperone activities as prerequisites for PVA replication. We propose that this mechanism contributes to shifting potyvirus RNA from translation to replication.

Keywords: Coat protein regulation, potato virus A

*Presenting author

Protein determinants of Potyvirus translation

*Shreya Saha, Kristiina Mäkinen
University Of Helsinki, Finland

Potato virus A (PVA) is a single-stranded positive sense RNA virus, which belongs to the genus Potyvirus. Viral protein genome-linked (VPg) regulates PVA gene expression by boosting the accumulation of PVA RNA and the amounts of viral proteins. VPg-mediated enhancement of PVA gene expression requires PVA helper component-proteinase (HCpro) and the host proteins eukaryotic initiation factor 4E and (iso)4E (eIF4Es), ribosomal protein P0 and varicose .To further analyze the role of VPg-eIF4E interaction in potyviral translation, we mutated the eIF4E binding domain within PVA VPg (VPgmut). Expression of exogenous VPgmut did not increase wild type PVA gene expression, supporting the importance of VPg-eIF4E in virus-specific translation. Viral gene expression from PVA carrying the VPgmut gene in infected Nicotiana benthamiana (Nb) plants was severely impaired and this could not be rescued by ectopic expression of wild type VPg. Interestingly, ectopic co-expression of VPg, P0 and HCpro affects differently Rluc accumulation when the Rluc encoding gene is inserted between either P1 and HCpro or nuclear inclusion protein b (NIb) and coat protein (CP) genes, being more enhanced when expressed from NIb/CP position in the infectious PVA cDNA. The possible biological significance of this phenomenon requires further investigation. Our recent results show that HCpro and cylindrical inclusion protein (CI), the RNA helicase of PVA, are both associated with ribosomes in PVA infected Nb plants.Ectopic expression of VPg together with CI enhanced accumulation of virus-derived Renilla luciferase more than VPg alone suggesting that also CI participates in PVA translation. In our recent paper we proposed that CI may act together with HCPro and VPg-Pro to relieve repression of viral RNA translation in infected cells, which is an intriguing possibility that warrants further investigation.

Keywords: Potyvirus, VPg, translation, CI

*Presenting author

Host protein VARICOSE is a co-regulator of Potato virus A translation

*Maija Pollari, Kristiina Mäkinen
University of Helsinki, Finland

Potato virus A (PVA) is a positive sense ssRNA virus belonging to genus Potyvirus, a diverse group of plant pathogens causing significant crop losses worldwide. We aim to uncover molecular mechanisms forming the foundations of PVA infections. Viral RNA (vRNA) is protected from the host's defenses by the main viral silencing suppressor HCpro which induces the formation of Potyvirus-induced granules (PGs). PGs contain, in addition to vRNA and proteins, host factors involved in fine-tuning the infection. VARICOSE, a WD-40 domain protein participating in mRNA decapping, was one of the host proteins found to be important for PGs as the silencing of VARICOSE genes reduced PG formation. In addition the lack of VARICOSE severely diminished PVA translation (Hafrén et al 2015).

Nicotiana benthamiana has three homologous VARICOSE genes, which were tagged with a fluorescent protein and expressed ectopically as a mixture. We used confocal microscopy to confirm the presence of VARICOSE proteins in PGs. HCpro efficiently sequesters VARICOSEs into PGs resulting in near complete colocalization.

While overexpression of the VARICOSEs had a small positive effect on PVA translation, a more substantial boost was observed when they were coexpressed with viral protein genome-linked (VPg), a key player targeting viral RNA to active translation. The result is in line with previous observations that the silencing of VARICOSEs prevents VPg-mediated translational enhancement. VPg requires HCpro to boost PVA translation but surprisingly VARICOSE overexpression enabled VPg to escalate translation to some extent even in the absence of HCpro.

While being an integral part of PGs, which can protect vRNA against antiviral silencing mechanisms, host VARICOSE proteins also stimulate PVA translation together with VPg. Thus, they appear to have a co-regulatory role in establishing a successful PVA infection.

Keywords: VARICOSE, Potyviruses, translation, host-virus interactions

*Presenting author

Isolation and characterization of new viruses infecting halophilic archaea

*Tatiana Demina[1], Nina Atanasova[1], Maija Pietilä[1], Julija Svirskaite[1], Janne Ravantti[1], Andrius Buivydas[2], Dennis Bamford[1], Hanna Oksanen[1]
[1]University of Helsinki, Finland
[2]University of Groningen, Netherlands

According to the hypothesis of structure-based viral lineages, all viruses could be efficiently classified based on their virion architecture [1]. To test this hypothesis, more virus-host systems should be isolated and characterized. Here, we used a solar saltern in Thailand as a source of new viruses. We isolated 36 new archaeal viruses from salt samples, increasing the total number of known archaeal viruses to about 140 [2]. The obtained viruses displayed four morphotypes: myovirus-like (27 isolates), siphovirus-like (4), pleomorphic (4), and tailless icosahedral (1). Two viruses, Haloarcula californiae icosahedral virus 1 (HCIV-1) and Haloarcula hispanica pleomorphic virus 3 (HHPV3), were characterized in detail. The HCIV-1 icosahedral virions, 70 nm in diameter, possess a lipid membrane underneath a protein shell. Virus genome is a 31,314 bp-long linear dsDNA with 47 genes/ORFs [3]. HHPV3 virions are pleomorphic lipid vesicles of 50 nm in diameter decorated by spike proteins. The HHPV3 genome is a 11,648 bp-long circular dsDNA containing 17 genes/ORFs [4]. HCIV-1 characteristics suggest that it belongs to the same group as three other tailless icosahedral haloarchaeal viruses – SH1, PH1, and HHIV-2 – all isolated from distant hypersaline environments. In terms of virion architecture, these viruses and HCIV-1 belong to the PRD1-adenovirus lineage, which comprises dsDNA tailless icosahedral viruses infecting hosts from all three domains of life. The revealed properties of HHPV3 suggest that the virus belongs to a word-wide distributed group of pleomorphic viruses infecting haloarchaea. The results of this study are consistent with the observation that a limited number of known virus morphotypes are found all over the biosphere, supporting the hypothesis of structure-based viral lineages.

1. Bamford et al. Theor. Popul. Biol. 61, 461-470 (2002).

2. Atanasova et al. Viruses 7, 1902-1926 (2015).

3. Demina et al. mBio 7 (2016).

4. Demina et al. Virology 499, 40-51 (2016).

Keywords: archaeal viruses, hypersaline environment, structure-based viral lineages

*Presenting author

Transmission electron microscopy investigation of capsid breakage and formation of protein aggregates during adenoviral vector manufacturing

*Vesa Turkki [1], Janne Weisell [1], Piia Käyhty [1], Gustaf Kylberg [2], Martin Ryner [2], Johan Harmark [2], Minna Hassinen [1], Hanna Lesch [1], Minna Karhinen [1], Robert Shaw [3], Seppo Ylä-Herttuala [4]
[1]FinVector Vision Therapies Oy, Finland
[2]Vironova AB, Sweden
[3]Trizell Ltd, United Kingdom
[4]A.I. Virtanen Institute for Molecular Sciences, University of Eastern Finland, Finland

Adenoviruses are among the most commonly used vectors for gene therapy. Large-scale manufacturing processes have been developed for the production of high-titer adenoviral vectors. Not all adenoviral structural proteins are incorporated into virions during the upstream production phase and additional viral proteins are released if vector particles are broken during the process. These proteins, unless removed by the downstream purification steps, can aggregate or promote aggregation of vector particles.

This study explored the capability of transmission electron microscopy to detect viral structural protein aggregates, free capsomers and broken viruses in samples from clinical grade adenovirus downstream processing. The study relied heavily on the use of Mini-TEM transmission electron microscope, which allows automated image acquisition. In addition the system can count the number of viral particles and debris in the obtained images. Automated operation of the system reduced the operator hands-on time and enabled us to obtain a large number of images for subsequent analysis.

Development of algorithms is ongoing but currently these analysis have allowed us to observe changes in adenovirus vector product homogeneity and purity during the downstream processing. Microscopy results can be linked to final product protein aggregate/particulate numbers, and the biochemistry and molecular biology results support the hypothesis about particulate identity. The obtained results will be valuable for future process development purposes.

Keywords: electron microscopy, protein aggregation, vector manufacturing, adenoviral vectors

*Presenting author

Asymmetric flow field flow fractionation methods for virus purification

Katri Eskelin, *__Mirka Lampi__, **Hanna Oksanen**, **Dennis Bamford**
University of Helsinki, Finland

Detailed biochemical and biophysical characterization of viruses requires viral preparations of high quantity and purity. Purification of viruses is challenging since viruses are fragile and may loose their functionality and biological activity in the process. The optimization of virus production and purification is an essential, but laborious and time-consuming process. Asymmetric flow field flow fractionation (AF4) provides an attractive alternative method for virus purification because it is a rapid and gentle separation method that generally preserves well biological functionality.

We have optimized the AF4 conditions to be used for purification of viruses with different morphologies, biochemical and –physical properties. Our results show that AF4 is well suited for virus purification as monitored by virus recovery and specific infectivity. Short analysis time and high sample loads enabled us to use AF4 for preparative scale purification of several viruses. Furthermore, we show that AF4 enables the rapid real-time analysis of progeny virus production in infected cells. The data presented here is from the purification of our model virus, bacteriophage PRD1. Nevertheless, AF4 method has proven to be applicable also to various different viruses, even for viruses having an outer lipid envelope. Here, with PRD1, we show that purification by AF4 results in virus preparations with high purity and infectivity. With AF4 purification from cell lysate we obtained virus yield and specific infectivity comparable to traditional purification methods (polyethylene glycol precipitation, rate-zonal and differential centrifugation). Consequently, AF4 provides a rapid, one-step method to produce virus material from crude cell lysates for initial biochemical and -physical characterization of viruses.

Keywords: asymmetric flow field flow fractionation, virus purification, PRD1

*Presenting author

High-resolution structure of lipid containing Thermus virus P23-77 using cryo-EM

*Anne B Ries, James A Geraets, Shabih Shakeel, Sarah J Butcher
University of Helsinki, Finland

Thermus virus P23-77 is the type species of the recently assigned genus Gammasphaerolipoviruses in the family of Sphaerolipoviridae, and infects the Gram negative, thermophilic bacterium Thermus thermophilus. Originally isolated from an alkaline hot spring in New Zealand, it is a 17.036 kbp dsDNA phage of spherical, tailless, spiked morphology with a capsid diameter of 78 nm. Between capsid and genome, the virus contains a lipid bilayer.

The P23-77 capsid follows a T=28d quasiequivalent icosahedral symmetry. It is made up of an unknown number of structural proteins, of which crystal structures are available for the major capsid proteins VP16 and VP17. VP16 is found at the vertices and icosahedral two-fold and three-fold axes, whereas VP17 is found around the vertices and two-fold axis. However, the identity of the core proteins of the vertices is unknown, and includes the interaction with the inner membrane. Therefore, we seek to gain atomic resolution maps that will allow the identification of the protein composition of the icosahedral five-fold axis.

Using cryo-EM and single particle analysis, we determined the structure of this bacteriophage to 4.3 Å by applying icosahedral symmetry during image processing. Imposing symmetry, however, degrades asymmetric information available in the structure. There is some weak density seen around the vertices: we will identify whether it belongs to the spike proteins and in order to resolve it we will utilize local reconstruction methods.

In summary, this work will provide a comprehensive view on the composition of an icosahedral virus capsid with a symmetry of rare occurrence.

Keywords: Thermus virus P23-77, cryo EM

*Presenting author

Optimized production and purification of Coxsackievirus B1 vaccine and its preclinical evaluation in a mouse model

*Minna Hankaniemi[1], Olli Laitinen[1], Virginia Stone[2], Amirbabak Sioofy-Khojine[1], Pär Larsson[2], Varpu Marjomäki[3], Heikki Hyöty[1], Malin Flodström-Tullberg[2], Vesa Hytönen[1]
[1]University of Tampere, Finland
[2]Karolinska Institutet, Sweden
[3]University of Jyväskylä, Finland

Coxsackie B viruses are among the most common enteroviruses, causing a wide range of diseases. Recent studies have also suggested that they may contribute to the development of type 1 diabetes. Vaccination would provide a highly effective way to prevent CVB infections, and the objective of this study was to develop an efficient vaccine production protocol for the production of novel CVB vaccines. Various steps in the production of a formalin-inactivated Coxsackievirus B1 (CVB1) vaccine were optimized including the Multiplicity Of Infection (MOI) used for virus amplification, virus cultivation time, type of cell growth medium, virus purification method and formulation of the purified virus. Safety and immunogenicity of the formalin inactivated CVB1 vaccine was characterized in a mouse model. Virus propagation in serum-free media in Vero cells was found to be an efficient method for the production of CVB1 viral particles. Two of the developed methods were found to be optimal for virus purification: the first employed PEG-precipitation followed by gelatin-chromatography and sucrose cushion pelleting (three-step protocol), yielding 83 fold increase in virus concentration. The second method utilized tandem sucrose pelleting without a PEG precipitation step, yielding 19 fold increase in virus yield, but it was more labor-intensive and cannot be efficiently scaled up. Both protocols provide radically higher virus yields compared with traditional virus purification protocols involving PEG-precipitation and sucrose gradient ultracentrifugation. Formalin inactivation of CVB1 produced a vaccine that induced a strong, virus-neutralizing antibody response in vaccinated mice. CVB1 viral particles for vaccine production can be efficiently produced using a serum-free Vero-cell based production system and an easily scalable three-step purification protocol. Moreover, the formalin inactivated CVB1 vaccine strongly induced virus-specific neutralizing antibodies in mice highlighting its efficaciousness without a need for adjuvant. Altogether, these results provide valuable information for the development of new enterovirus vaccines.

Keywords: Coxsackievirus B1, vaccine, virus purification, formalin inactivation

*Presenting author

Single-molecule measurements of viral ssRNA packaging

*Gabija Ziedaite-Ojala[1], Kalle Hanhijärvi[2], Dennis Bamford[3], Edward Haeggström[2], Minna Poranen[1]
[1]Department of Biosciences, University of Helsinki, Finland
[2]Department of Physics, University of Helsinki, Finland
[3]Department of Biosciences, and Institute of Biotechnology, University of Helsinki, Finland

Assembly of the virus particles is an essential aspect of the virus life cycle, ensuring the production of new infectious virions and subsequent spread of the virus. The most distinctive feature of dsRNA virus life cycle is that genome replication and transcription occurs inside an icosahedral protein shell, in order to prevent the potential dsRNA induced host responses. Therefore, the genome is packaged as positive-sense single-stranded RNA precursors and the negative-strands are synthesized inside the procapsid.

Genome packaging of double-stranded RNA phages has been widely studied, but the details of this process at single-molecule level are poorly understood. Our model system is Pseudomonas phage phi6 which is a dsRNA virus with a tripartite genome. As in other dsRNA viruses the genome of phi6 is always replicated and transcribed inside the core particle.

We present single-molecule measurements of the viral ssRNA packaging by the phi6 procapsid. To our knowledge, this is the first single-molecule measurement of viral ssRNA packaging using optical tweezers. Our results show that the mean RNA packaging velocity by phi6 is relatively low, however, packaging could reach (4.6 nm/s; 12.5 nt/s) during the fast packaging phase. The packaging proceeds intermittently in slow and fast phases. These slow and fast phases correspond to secondary structures in the RNA which is being packaged.

Keywords: RNA packaging, phage Phi6, Optical tweezers, dsRNA virus

*Presenting author

SHOULD SOLID STOOLS BE USED IN NOROVIRUS DIAGNOSTICS?

Juha M. Koskinen[1], *__Petri Susi__*[2], **Kaisu Rantakokko-Jalava**[3], **Marjo Salminen**[4], **Minna Paloniemi**[4], **Janne O. Koskinen**[1]
[1]ArcDia International Oy Ltd, Finland
[2]University of Turku, Finland
[3]Turku University Hospital, Finland
[4]University of Tampere, Finland

Human noroviruses are the leading cause of acute gastroenteritis outbreaks worldwide. Symptoms of norovirus infection vary a lot from symptomatic (e.g. diarrhoea, vomiting combined with nausea, abdominal cramps/pain, headache, chills and/or fever) to asymptomatic cases. In symptomatic norovirus infection diarrhoea may not always be present. In contrast, norovirus can be detected from stools irrespective of the consistency of the stool. In experimental infection studies it has been shown that norovirus can be detected with ELISA and RT- PCR in stool samples up to 1 and 4 weeks (median) after inoculation, respectively. We studied retrospectively the correlation between the consistency of stool, collected from symptomatic patients, and quantitative norovirus concentration. A new automated multi-analyte antigen test, the mariPOC gastro, which covers tests for norovirus GI and GII.4 was used for in vitro diagnostic testing. The test also detects rotavirus, adenovirus and Campylobacter spp. Norovirus positive stool samples were collected in Tyks Micorbiology and Genetics in winter 2015. Consistency of the stool samples was categorized as 1) liquid (watery diarrhoea), 2) semi-solid, 3) soft and 4) solid. As a proof of true positivity at least one out of five lateral flow tests (different manufacturers) and mariPOC norovirus GII.4 test or PCR/sequencing must coincide. Thirty two stools were true positives. Viral concentrations measured from the stools were blotted against the stool consistency. Differences in viral levels were not observed between different stool consistencies. The highest viral concentration that were found were at least 3x10E11 virus particles per gram of stool, whether the stool was liquid or solid. This study confirmed that noroviruses (GII.4) are shed in stool irrespective of the consistency of the stool during symptomatic infection. Therefore, in clinical practise also patients with solid stools and absence of diarrhoea can be tested for noroviruses if clinical findings otherwise may suggest gastrointestinal infection.

Keywords: norovirus, diagnostics, diarrhoea, stool

*Presenting author

Comparative diagnosis of human bocavirus 1 respiratory infection by mRNA RT-PCR, quantitative PCR and serology

*Man Xu[1], Benedict Arku[1], Tuomas Jartti[2], Janne Koskinen[3], Ville Peltola[2], Klaus Hedman[4], Maria Söderlund-Venermo[1]
[1]Department of Virology, University of Helsinki, Helsinki, Finland
[2]Department of Pediatrics and Adolescent Medicine, Turku University Hospital and University of Turku, Turku, Finland
[3]ArcDia International Oy Ltd, Turku, Finland
[4]University of Helsinki and Helsinki University Hospital, Helsinki, Finland

Background: Human bocavirus (HBoV) 1 can cause life-threatening respiratory tract infection in children. Diagnosing acute HBoV1 infection is challenging due to long-term airway persistence. We assessed whether messenger (m)RNA detection would correlate better than mere DNA detection, with acute HBoV1 infection.

Methods: Serial nasopharyngeal swab (NPS) and serum samples of 121 children with acute wheezing were analyzed by quantitative (q)PCR, reverse-transcription (RT)-PCR and serology.

Results: By serology, 16/121 (13.2%) children had acute HBoV1 infection, all of whom at the wheezing phase had in NPS HBoV1 DNA, whereas 12/16 (75%) had HBoV1 mRNA. Among 25 non-diagnostic children, 6 had in NPS HBoV1 DNA and 1 had mRNA. All 13 mRNA-positive samples exhibited high HBoV1 DNA loads of $\geq$106 copies/ml. No mRNA persisted for 2 weeks, whereas HBoV1 DNA persisted for two months in 4 children; one year later all 15/15 available samples were DNA negative. Compared to serology, DNA PCR had high clinical sensitivity (100%) but, due to virus persistence, low specificity (76%). In contrast, mRNA RT-PCR had low clinical sensitivity (75%), but high specificity (96%).

Conclusions: A combination of HBoV1 serology, DNA qPCR and mRNA RT-PCR should be employed for accurate diagnosis of HBoV1 infection.

Keywords: human bocavirus 1, respiratory tract infection, diagnostic methods, wheezing children

*Presenting author

Multiplex detection in tonsillar tissue of all human polyomaviruses known

Mohammadreza Sadeghi[1], *__Yilin Wang__[1], **Torbjörn Ramqvist**[2], **Leena-Maija Aaltonen**[3], **Lari Pyöriä**[1], **Mari Toppinen**[1], **Maria Söderlund-Venermo**[1], **Klaus Hedman**[4]
[1]Virology, University of Helsinki, Finland
[2]Department of Oncology-Pathology, Karolinska Institutet, Stockholm, Sweden
[3]Department of Otorhinolaryngology-Head and Neck Surgery, Helsinki University Hospital; and University of Helsinki, Finland
[4]Virology, University of Helsinki; and Helsinki University Hospital, HUSLAB, Finland

Background: In the past few years, eleven new members have joined the two previously known members JCPyV and BKPyV of the Polyomaviridae family, by virtue of molecular methods. Serology data suggest that infections with human polyomaviruses (HPyVs) occur since childhood and the viruses are widespread in the general population. However, the viral persistence sites and transmission routes are by and large unknown. Our previous studies demonstrated that the four new HPyVs – KIPyV, WUPyV, MCPyV and TSPyV – were present in the tonsils, and suggested lymphoid tissue as a latency site of these emerging human viruses.

We developed a Luminex-based multiplex assay for simultaneous detection of all 13 HPyVs known, and explored their occurrence in tonsillar tissues of children and adults mostly with tonsillitis or tonsillar hypertrophy.

Methods: We set up and validated a new Luminex-based multiplex assay by using primer pairs and probes targeting the respective HPyV viral protein 1 (VP1) genes. With this assay we tested 78 tonsillar tissues for DNAs of 13 HPyVs.

Results: The multiplex assay allowed for simultaneous detection of 13 HPyVs with high analytical sensitivity and specificity, with detection limits of 100-102 copies per microliter, and identified correctly all 13 target sequences with no cross reactions. HPyV DNA altogether was found in 14 (17.9 %) of 78 tonsils. The most prevalent HPyVs were HPyV6 (7.7%), TSPyV (3.8%) and WUPyV (3.8%). Mixed infection of two HPyVs occurred in one sample.

Conclusions: The Luminex-based HPyV multiplex assay appears highly suitable for clinical diagnostic purposes and large-scale epidemiological studies. Additional evidence was acquired that the lymphoid system plays a role in HPyV infection and persistence. Thereby, shedding from this site during reactivation might take part in transmission of the newly found HPyVs.

Keywords: HPyV, tonsil, Luminex, PCR

*Presenting author

Innate Immune Responses to Clinical Herpes Simplex Virus Isolates in Nervous System-Derived Cells

*Jenni Lehtinen, Henrik Paavilainen, Veijo Hukkanen
University of Turku, Finland

Herpes simplex virus (HSV) is a neurotropic virus and has recently become the pioneering oncolytic virotherapy. The knowledge on the innate immune responses, induced by circulating clinical HSV strains, is vital for understanding the pathogenesis of HSV, as well as for the development of vaccines and HSV-based virotherapies. We have studied the innate immunity induction by low-passage HSV-1 and HSV-2 field isolates, derived from clinical specimens, in cell lines representing the natural host tissues of HSV. We included laboratory wt strains of HSV-1 (17+) and 2 (G) in our studies as references. The growth properties, plaque morphology, and sensitivity for aciclovir were also studied. We used HaCaT epithelial cells, U373MG astrocytoma and T98G glioma cells, and SH-SY5Y neuroblastoma cells for the study. We evaluated the ability of each cell line to express type I or III interferons and interferon-stimulated genes after stimulation with double-stranded RNA.

HSV-1 strains showed more prominent replication in these human cell lines compared to HSV-2 strains, except in T98G glioma cells, in which HSV-2 strains replicated stronger. HSV-2 strains also induced stronger interferon alfa, beta, lambda1 (IL-29), PKR and ISG54 responses in T98G than did the HSV-1 strains. Clinical HSV isolates had diverse profiles in viral shedding and varying immunostimulatory properties. Although the wt strain HSV-1 (17+) replicated most efficiently, the innate responses it induced were modest in comparison with those to clinical HSV-1 isolates. One HSV-1 isolate strain induced especially strong type I and type III interferon responses in U373MG cells. Our aim is to develop gene therapy vectors for treatment of cancers of the nervous system, derived from selected clinical HSV isolates. Based on our studies, HSV-2 strains seem to be more immunogenic and suitable for oncolytic immuno-virotherapies of glioma than HSV-1 strains.

Keywords: innate immune responses, herpes simplex virus, clinical isolates

*Presenting author

Coxsackie B viruses are detected more frequently in serum samples of children at risk for developing type 1 diabetes than in healthy controls

*Hanna Honkanen[1], Sami Oikarinen[1], Olli Laitinen[2], Minna Hankaniemi[2], Jorma Ilonen[3], Olli Simell[3], Jorma Toppari[3], Riitta Veijola[4], Mikael Knip[5], Heikki Hyöty[1]
[1]University of Tampere, Finland
[2]University of Tampere, Vactech Ltd., Finland
[3]University of Turku, University hospital of Turku, Finland
[4]University of Oulu, University hospital of Oulu, Finland
[5]University of Helsinki, University hospital of Helsinki, University hospital of Tampere, Finland

Enteroviruses are considered as the prime candidates for viral triggers of islet autoimmunity in type 1 diabetes. Our previous studies have demonstrated that detection of enteroviruses RNA in blood and in stool samples precedes the detection of diabetes associated islet autoantibodies. In addition, two independent studies showed that increased risk of islet autoimmunity was associated with a narrow phylogenetic group of enteroviruses, group B coxsackieviruses (CVBs), by analyzing the neutralizing antibodies against these viruses. The main risk virus was CVB1. The aim of this study was to analyze the prevalence and types of acute enterovirus infections in serum samples and in stool samples by RT-PCR. The other aim was to analyze whether particular enterovirus types could be linked to the pathogenesis of type 1 diabetes. Serum and stool samples were collected from children participating in the Diabetes prediction and prevention (DIPP) study in Finland. 5695 serum samples and 4781 stool samples were analyzed for the presence of enterovirus RNA by RT-PCR. Enterovirus types were identified by sequencing the VP1 region of the genome. The prevalence of enterovirus RNA in serum samples was 2.0% compared to 7.7% in stool samples. The prevalence of enteroviruses did not differ between case children and control children in either of the sample type. The sequence analysis revealed 25 different enterovirus types in stool samples and 12 types in serum samples. None of these were associated with the islet autoimmunity. Group A coxsackieviruses dominated in both sample types. Overall, the prevalence of coxsackie B viruses was higher in serum samples than in stool samples and group B coxsackieviruses tended to be more frequent in serum samples of children at risk for type 1 diabetes than in healthy control children. This trend supports earlier findings of the risk association of group B coxsackieviruses in type 1 diabetes.

Keywords: coxsackie B viruses, RT-PCR, enteroviruses

*Presenting author

generation of pseudoviruses containing reptarenavirus glycoproteins in replication-defective recombinant Vesicular stomatitis virus

*Yegor Korzyukov[1], Jussi Hepojoki[2], Rommel Iheozor-Ejiofor[3], Olli Vapalahti[2]
[1]University of Helsinki, USA
[2]University of Helsinki, Finland
[3]University of Helsinki, United Kingdom

The family of Arenaviridae comprise two genera, Mammarenavirus and Reptarenavirus. Most mammarenaviruses are rodent-borne, and some can cause severe and sometimes fatal hemorrhagic fever in humans.

Reptarenaviruses are linked to boid inclusion body disease (BIBD) that occurs in captive snakes. The pathognomonic hallmark of BIBD is the formation of electron-dense inclusion bodies (IB). The IB are found in practically all cell types. The signs of BIBD include neurological disturbances, abnormal skin shedding, head tremors, and regurgitation.

Arenaviruses genome is bi-segmented, large (L) and small (S), negative stranded RNA. The L segment encodes RNA-dependent-RNA-polymerase (RdRp) and a small zinc-finger protein (Z). The S segment encodes viral nucleocapsid protein (NP) and glycoprotein precursor (GPC) that after proteolytic processing yields stable signal peptide (SSP), GP1 and GP2.

We have shown that reptarenaviruses can infect mammalian, reptilian and even arthropod cell lines. Mammarenaviruses use either transferrin receptor 1 or a-dystroglycan as their cellular receptor, however the receptor(s) of reptarenaviruses are unknown.

Pseudovirus production using replication-defective recombinant Vesicular stomatitis virus (VSV) system has been utilized before with other viruses, however it hasn't been used with reptarenviruses.

We have generated pseudotyped VSVs with GFP marker for the study of reptarenavirus receptors in cell culture. We cloned the GPC of University of Helsinki Virus-1 (UHV-1) and University of Giessen-1 (UGV-1) into pCAGGS vector. We used human embryonic kidney cells (HEK293) and boa kidney cells (I/1Ki) for expression of the GPCs from pCAGGS vector. We then infected the transfected cells with recombinant VSV ΔG (rVSV- ΔG), which includes the GFP marker. The rescued pseudotype viruses were recovered from cell culture medium and used for infection of mammalian and reptilian cells. Here we demonstrate, that rVSV- ΔG can be used with reptarenavirus GPC to produce pseudoviruses.

Keywords: vsv, reptarenavirus, snake, receptor

*Presenting author

Arboviruses in Kenya

Moses Masika[1], *__Essi Korhonen__*[2], **Lorna Culverwell**[2], **Olli Vapalahti**[2], **Eili Huhtamo**[2]
[1]University of Nairobi, Kenya
[2]University of Helsinki, Finland

Many mosquito-borne viruses (MBVs) originate in tropical Africa, where there is both high wildlife reservoir and vector species diversity in close vicinity of human habitation. In Africa, the lack of resources for specific laboratory diagnostics is likely to result in underestimates of the impact of MBVs to public health. Additionally, the number of detected human cases is often a fraction of those actually transmitted; therefore surveys of wildlife (vectors and potential vertebrate hosts) are needed to obtain reliable distribution data for MBVs.

We aim to obtain information on the prevalence and diversity of MBVs and their mosquito vector species in Kenya in a study based on University of Helsinki field station Taita. We have collected mosquitoes (several thousands) and human samples from febrile patients (n=358) from Wundanyi and Mombasa areas in years 2015-2016 (additional collections will still take place in spring 2017) which will be screened for MBV nucleic acids and antibodies respectively. The obtained data will be combined to form distribution information, and using the GIS system, also models enabling predictions of risk areas for MBV transmission. The preliminary results of serological tests suggest flavi- and alphavirus circulation in the studied areas.

Keywords: mosquito-borne viruses, arboviruses

*Presenting author

Extracellular vesicles can deliver oncolytic adenoviruses to cancer cells

*Katariina Koskinen[1], Heikki Saari[2], Mariangela Garofalo[2], Petter Somersalo[2], Laura Aksela[2], Vincenzo Cerullo[2], Lukasz Kuryk[2], Sari Mattila[1], Marjo Yliperttula[2], Matti Jalasvuori[1]
[1]Department of Biological and Environmental Science, Nanoscience Center, University of Jyväskylä, Finland
[2]Division of Pharmaceutical Biosciences, Centre for Drug Research, University of Helsinki, Finland

Abstract

Cells are known to produce external vesicles that mediate intercellular transfer of various types of biomolecules. Interestingly, cells infected with viruses, which are traditionally considered to be non-enveloped (such as adenoviruses), generate extracellular vesicles (EVs) that have entrapped virus particles inside. As these vesicles fuse with other (non-infected) cells, they deliver their viral cargo in a receptor-independent manner. Our experiments suggest that EV-virus complexes produced in cancerous cell lines induce apoptotic phenotype in similar cancer cells already two- hours post-infection. Further, intratumourally and intravenously introduced EV-viruses (along with EV-virus-chemo combinations) were shown to limit the growth of tumors in a mouse cancer model, hence EVs could possibly serve as drug-delivery vessels for novel cancer therapies. On a more speculative tone, it is also conceivable that EVs play a (critical) role in the viral within-host life cycle by providing the virus a camouflage against host immune system in the extracellular environment while carrying the virus in tissue-specific fashion between individual cells.

Keywords: cancer, adenovirus, extracellular vesicles

*Presenting author

Promoter targeted histone acetylation of chromatinized parvoviral genome is essential for infection progress

*Elina Mäntylä[1], Kari Salokas[2], Mikko Oittinen[3], Vesa Aho[4], Pekka Mäntysaari[1], Lassi Palmujoki[1], Olli Kalliolinna[1], Teemu Ihalainen[5], Einari A. Niskanen[6], Jussi Timonen[7], Keijo Viiri[3], Maija Vihinen-Ranta[1]
[1]Department of Biological and Environmental Science and Nanoscience Center, University of Jyväskylä, Finland
[2]University of Helsinki, Finland
[3]Tampere center for child health research, University of Tampere, Finland
[4]Department of Physics and Nanoscience Center, University of Jyväskylä, Finland
[5]NeuroGroup, BioMediTech, University of Tampere, Finland
[6]Institute of Biomedicine, University of Eastern Finland, Finland
[7]Department of Physics and Nanoscience Center,University of Jyväskylä, Finland

The association of host histones with parvoviral DNA is poorly understood. We analyzed the chromatinization and histone acetylation of canine parvovirus DNA during infection by confocal imaging and in situ proximity ligation assay combined with chromatin immunoprecipitation and high-throughput sequencing. We found that at late infection parvovirus replication bodies were rich in histones bearing modifications characteristic of transcriptionally active chromatin, i.e. histone H3 lysine 27 acetylation (H3K27ac). The H3K27ac, in particular, was located in close proximity to the viral DNA-binding protein NS1. Importantly, our results show for the first time that in the chromatinized parvoviral genome, particularly the two viral promoters were rich in H3K27ac. Histone acetyltransferase (HAT) inhibitor efficiently interfered with expression of viral proteins and infection progress. Altogether, our data suggest that acetylation of histones on parvoviral DNA is essential for viral gene expression and completion of viral life cycle.

Keywords: parvoviral genome, histone acetylation, Epigenetic regulation, chromatinization

*Presenting author

Chromatin organization regulates viral egress dynamics

*Vesa Aho [1], Markko Myllys [1], Visa Ruokolainen [1], Satu Hakanen [1], Elina Mäntylä [1], Carolyn Larabell [2], Maija Vihinen-Ranta [1]
[1]University of Jyväskylä, Finland
[2]Lawrence Berkeley National Laboratory, USA

Various types of DNA viruses are known to elicit the formation of a large nuclear viral replication compartment and marginalization of the cell chromatin. We used three-dimensional soft x-ray tomography, confocal and electron microscopy, combined with numerical modelling of capsid diffusion to analyse the molecular organization of chromatin in herpes simplex virus 1 infection and its effect on the transport of progeny viral capsids to the nuclear envelope. Our data showed that the formation of the viral replication compartment at late infection resulted in the enrichment of heterochromatin in the nuclear periphery accompanied by the compaction of chromatin. Random walk modelling of herpes simplex virus 1–sized particles in a three-dimensional soft x-ray tomography reconstruction of an infected cell nucleus demonstrated that the peripheral, compacted chromatin restricts viral capsid diffusion, but due to interchromatin channels capsids are able to reach the nuclear envelope, the site of their nuclear egress.

Keywords: capsid transport, herpesvirus, chromatin marginalization, numerical modeling

*Presenting author

IN VITRO CONDITIONS AFFECTING TO UNCOATING OF ECHOVIRUS 1

*Visa Ruokolainen, Varpu Marjomäki
University of Jyväskylä, Finland

Very recently, our group identified a novel type of infectious Echovirus 1 (E1) particle population during infection, a novel open infectious form of E1 (Myllynen et al., 2016, JVI spotlight). This novel particle is denser in CsCl gradient, permeable to SYBR Green II, and in contrast to for example poliovirus, it still contains most of its VP4 can bind to its receptor and show high infectivity. These results thus suggest that among enteroviruses there may exist different mechanisms on virus uncoating.

Here we focused on the effect of key ions (Na+, Cl-, Mg2+, Ca2+ and K+) and explored their direct effect on the virus particle. The formation of porous, SYBR Green II permeable virus particle and RNA release was studied by using real-time spectroscopy and radioative gradients. We hypothesized, that since the uncoating of E1 is not induced by low pH, and virus is not accumulating in acidic endosomes, other ions might contribute to opening of the virus capsid.

Our results show that several ion concentrations e.g. high Mg2+, Ca2+ and low K+ seemed to have a protective role in virus opening. In contrast, high NaCl concentration as such and low NaCl with high K+ seemed to open the structure. Even if some of the tested ionic conditions were promoting the formation of the porous intermediate E1 particle, the virions seemed to retain rather high infectivity suggesting that further cues in cells may be participating in efficient genome release.

This study provides further knowledge about the uncoating and genome release of E1. New information on the uncoating of enterovirus B group is needed since several details leading to the uncoating of E1 differs remarkably from the model enterovirus species poliovirus.

Keywords: Uncoating, In vitro, Ions, Echovirus 1

*Presenting author