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ORAL/TALK

Identification of membrane proteins from the aphid *Myzuspersicae*'s gut implicated in polerovirus transmission

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Plant viruses are responsible for important worldwide economic losses. Since most phytoviruses are transmitted from plant to plant by vectors, chemical treatments are applied to reduce vector populationsand to limit virus impact. Nevertheless, these methods are damageable for the environment and human health,and it iscrucial to elaborate alternativestrategiesto reduce virus transmission. In our laboratory, we study poleroviruses, single stranded RNA viruses transmitted by the aphid *Myzus persicae*in a persistent and circulative manner.

Virus particles, acquired by aphids on infected plants, cross intestinal cells by transcytosis to be released in the hemolymph. From there, they reach the accessory salivary gland cellsand are transported through the cell by a similar mechanism. Particles are then released, together with saliva, into a plant during aphid feeding. Virus transport through the epithelia relies on the presence of specific virus receptors that are still unknown. In order to identify these receptors, virus partners identification was previously conducted by screening an aphid cDNA library with virus structural proteins using the yeast two hybrid system andthree candidate proteins were identified. To increase the number of candidates, Iperformedin silico analyses ofRNAseq data and identified a list of XX membranous intestinal proteins that may have receptor activity. We are now developingRNAi-based strategies to inhibit expression of the candidate genes in the aphid and evaluate their implication in virus transmission. The functional validation is based on the oral acquisition by aphids of double stranded RNA synthesized in plant or *in vitro*.This project could lead to the identification ofaphid proteins involved in virus transmission but also proteins that ensure a vital function in the aphids. Inhibiting the expression of these proteins may ultimately result in the development of transgenic plants inhibiting virus transmission or displaying an insecticide effect.

The role of *sel-10* (Fwb7) in transdifferentiation

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Transdifferentiation (TD) is a process during which one differentiated cell changes its identity and become another specialized cell type. In our laboratory, we study a well-characterized single cell transdifferentiation event which occurs naturally in the rectum of the worm *Caenorhabditis elegans*. *C.elegans* has an invariant cell lineage. Each wild type worm process the same divisions at the same time. This particularity allows us to follow one of the 6 rectal cells, named Y, which transdifferentiates into the PDA motor neuron (Y-to-PDA). This mechanism begins in Y at the L_1 larval stage and is completed at the L_3 larval stage (around 9 hours are needed for the entire process).

At this occasion, I present our study on *sel-10* (Fbw7) which is an ubiquitin ligase E3 involved in Y-to-PDA transdifferentiation. We found that *sel-10* is genetically interacting with an histone demethylase (JMJD-3.1) which also have a role positive role during TD. JMJD-3.1 is an H3K27 demethylase which is correlate with a transcription activity.

One model is that *SEL-10* ubiquitinates a substrate which acts as a probable repressor of Y-to-PDA TD, triggering its degradation by the proteasome. To test this hypothesis, we are examining which SCF complex is involved in Y transdifferentiation. In addition, we have set up different strategies, using genetics and RNAi screening approaches, to identify SEL-10 substrate(s). We also asses the question of the proteasome role in the transdifferenciation using genetic and RNAi tools. We will thus report on our progresses at the meeting.

The cGAS-STING pathway exhibits antiviral activity against Hepatitis B virus infection

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Chronic hepatitis B virus (HBV) infection is a major health problem and a leading cause of hepatocellular carcinoma worldwide. The mechanisms of viral genome recognition by the host cell sensors and the induction of HBV-induced innate immune response are still poorly understood. Recently, the cyclic GMP-AMP synthase (cGAS) was reported as a cytoplasmic DNA sensor involved in sensing of different viruses. cGAS binds to doublestranded DNAs inducing the production of cyclic guanine-adenine dinucleotide (cGAMP) which is recognized by STING (Stimulation of interferon genes) triggering the production of type I interferons and antiviral ISGs (interferon stimulated genes). In this study, we aimed to understand the functional role of cGAS and STING-dependent antiviral pathways for HBV infection. We established HBV permissive cell culture model and we performed loss- and gain-of-function studies using RNAi (RNA interference), overexpression and CRISPR/Cas9 approach. We analyzed HBV-induced transcriptional reprogramming of the cGAS/STING pathway gene expression in time course experiments using RNASeq. We observed that silencing of cGAS, STING and TBK1 expression results in a robust an increase of HBV infection in HepG2-NTCP cells. In contrast, silencing of IFI16 gene expression (another cytoplasmic DNA sensor able to directly activate STING) had no effect on viral infection. Using a CRISPR/Cas9 gene knock-out approach, we confirmed that absent or reduced cGAS expression resulted in enhancement of HBV infection and replication. Finally, we observed that cGAS overexpression led to a significant decrease of HBV infection. Analysis of gene expression using qRT-PCR revealed that HBV infection suppressed STING and TBK1 gene expression in infected HepG2-NTCP cells. Time course experiments of infected cells analyzed by RNAseq showed that cGAS/STING signatures are highly modulated by HBV in a complex manner and our analyses identified several clusters of genes in the cGAS/STING signatures with specific responses to HBV infection at each timepoint and across the time course. Our loss- and gain-of-function studies using RNAi and CRISPR/Cas9 suggest a functional role for cGAS/STING-induced antiviral pathways in sensing and control of HBV infection. These findings reveal a potential mechanistic role of this pathway for viral evasion from innate immune responses and may open a perspective for new antiviral approaches exploiting viral sensing pathways.

Notes or short references

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Inhibition of the first HIV infection by antibodies in cervico-vaginal mucosa.

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HIV heterosexual transmission mostly affects women. Free virus and/or infected cells as CD4(+) T cells present in the seminal fluid infect mucosal target cells, e.g., macrophages, dendritic cells (DC), in addition to CD4(+) T cells. To define the first steps leading to HIV sexual transmission, we analyzed the virus transfer between HIV-infected CD4(+) T cells of a donor A and DC or CD4(+) T cells of a receiver B. We developed a more physiological model to study HIV target cells by isolating mucosal cells from cervico-vaginal tissues, and analyzed the kinetic of infection in the different cellular populations. Finally, inhibition mechanisms of target cell infection using different antibodies will be defined.

In vitro, we used an HIV transmission model, where primary CD4(+) T cells of a donor A infected with HIV-1 isolates at different times, are co-cultivated with DC and CD4(+) T cells of a donor B. Infection model of mucosal cells to different tissues (Fallop tubes, uterus, endocervix and cervix) was developed. These samples, obtained from HIV-seronegative volunteers' women, were provided by the "Centre Médico-chirurgical Obstétrique de Strasbourg". Tissues are dissociated using collagenase, infected with free virus or infected cells. Neutralizing antibodies are added in the different models to evaluate their inhibition capacity. Different cells are phenotyped and the percentage of infected cells in each cellular population are defined by intracellular staining of HIV viral protein p24 and analyzed by flow cytometer.

In these cases of allogenic transfer, we observed HIV transmission between CD4(+) T cells of A and DC and CD4(+) T cells of B after 24h post-infection. Interestingly, viruses are transferred first to DC, then to CD4(+) T cells. In the presence of antibodies, we observed a dose-dependent decrease of infected cells affecting both cell types of donor B. Regarding the tissues, we found a majority of lymphocytes in fallop tubes. In cervix, macrophages (CD64+) are predominant and in endocervix, two antigen-presenting cell types, DC (CD141+, CD1c+) and macrophages (CD64+) are more represented in addition to lymphocytes. 48h post-infection, we observed infected DC (CD141+, CD1c+) although those are less represented than lymphocytes in our cultures. These data suggest that DC would be the first infected cells. Further infection kinetics are currently being assessed in different tissues to define the first targets of HIV infection in the mucosa. Infection in presence or in absence of different antibodies, will define inhibition mechanism in the different HIV target cells in tissues.

All of these studies define the key role of antigen-presenting cells in mucosal HIV sexual transmission. Inhibition mechanisms, using antibodies, for HIV infection in different target cells in tissues would help to determine which antibodies should be induced by vaccination to prevent HIV sexual transmission in the mucosa.

A mitochondrially targeted CRISPR/Cas9 system as a potential way to treat mitochondrial DNA mutations disorders

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Mitochondria are essential organelles of eukaryotic cells since they produce most of their energy supply across oxydative phosphorylation which takes place in the mitochondrial respiratory chain. They possess their own genome in multiple copies, which in human is devoted to the production of 13 essential proteins of this respiratory chain. More than 300 pathogenic mutations of the mitochondrial DNA (mtDNA) have been identified, often associated with severe multi-systemic disorders characterized by an incidence of 1/5,000 people, however, no efficient treatment have yet been proposed [1]. Most of these mtDNA mutations are “heteroplasmic”, which means that mutant and wild-type genomes are simultaneously present in a same cell. The ratio between the two types of molecules, the so-called “heteroplasmy level” determines the appearance and severity of respiratory chain deficiencies and associated symptoms. The modulation of this heteroplasmy level could thus represent a potential therapeutic approach. Among the strategies which have been proposed, one consists in a selective inhibition of the mutant mitochondrial genomes replication [2,3], another, in a specific degradation of mutant mtDNA molecules [4,5]. Our laboratory focuses on the mechanisms of RNA import into mitochondria and try to use these pathways in order to develop therapeutic approaches.

The CRISPR/Cas9 system is a powerful genome editing technology which enables a double-stranded cleavage of DNA at a locus of interest by an RNA-guided endonuclease called Cas9. This system has never been adapted for an application on the mitochondrial genome. Our goal is then to address it into mitochondria in order to cleave specifically the mutant mtDNA and therefore, to induce a shift of the heteroplasmy level. Recently, by using various import determinants we succeeded to design and to import into human mitochondria, 1) specific gRNAs targeting the mtDNA and 2) the endonuclease Cas9. Applying this importable CRISPR/Cas9 to cultured human cells, we observed important depletion of mtDNA suggesting that this system is active in mitochondria. To optimize our system, we created a stable cell line expressing in an inducible way the importable Cas9 protein. We also designed and produced gRNAs targeting specifically a mutant mtDNA affected by a large deletion associated with a Kearns Sayre Syndrome. Their import and ability to cleave selectively mutant mtDNA molecules have been demonstrated *in vitro*. Application on *trans*mitochondrial human cells is now in progress.

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A new role for retinal ephrin-As in topographic map formation

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Accurate processing of the sensory stimuli relies on neural maps present throughout the brain. The superficial layers of the superior colliculus (SC) in the midbrain receive projections from retinal ganglion cells (RGCs) and V1 cortex that are aligned and in register, forming a visuotopic map. The molecular mechanisms of visuotopic map alignment in the SC are unknown. We describe a new mouse model, the Isl2-ephrin-A3, wherein ephrin-A3 expression is specifically elevated in an alternating subset of RGCs, producing two mixed populations of cells with distinct levels of ephrin-As. We find that these populations form a single coherent map in the SC, demonstrating that retinal ephrin-A3 is not directly involved in retino-collicular mapping. Unexpectedly, V1 cortico- collicular projections are duplicated in Isl2-ephrin-A3 animals, leading to a visuotopic mismatch in the SC. This is further confirmed when in vivo inactivation of retinal ephrin-A3 restores a single wild-type cortico-collicular map. Our data strongly suggest that retinal ephrin-A3, carried to the SC by RGC axons, provides positional information for ingrowing cortical axons. This work identifies a new molecular mechanism supporting the retinal-matching model of visuotopic map formation and alignment in the midbrain.