Incoming from Netherland

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Education

Proto-EpiFluist

BSc: Biology & Science Education (double major)

MSc: Molecular Cell Biology (2003)

1e PhD: Microbiology (2008)

2e PhD: Virus-Host Interaction, Biochemistry (2012)

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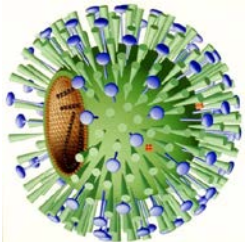
Agreenskills Scientist Mobility Programme

EPIGENETICS OF INFLUENZA VIRUS INFECTIONS



What is Epigenetics?

Wɔt ɪz ɛpɪdʒɪˈnɛtɪks/?

The term epigenetics refers to heritable changes in gene expression (active versus inactive genes) that does not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype.

- **Current known systems considered to initiate and sustain epigenetic changes are regulated by DNA methylations, histone modifications and non-coding RNA molecules**

- **All mentioned epigenetic chances regulate gene expression at the transcriptional and/or post-transcriptional level and involving in epigenetic processes such as DNA methylation targeting, heterochromatin formation, histone modification and gene silencing.**

Influenza A virus (IAV)

belongs to orthomyxoviruses

- **Annual influenza epidemics cause 3-5 million cases of severe illness and 250-500 thousand deaths every year around the world in human population and important losses in the poultry industry.**
- **Most notorious Influenza H1N1 pandemics was the Spanish flu which killed millions of people in 1918-1920.**
- **More recent outbreaks were an avian strain H5N1 which caused Bird Flu in 2004. The novel flu strain H1N1 which caused Swine Flu in 2009, which evolved from combined genes from human, pig, and bird flu. Both viruses can transfer from animal to human, but do not spread easily between humans yet.**

PROJECT - Rationale and Objectives

Understanding of epigenetic mechanisms in relation to infection diseases is crucial before any disease prediction, prevention and effective drug design strategies.
In Influenza the consequences of epigenetic processes are still under discussion.

Secondly, influenza viruses are masters at circumventing their hosts' defenses. They co-opt cellular protein-synthesis pathways to produce viral proteins. By hijacking this regulatory machinery, the virus inhibits the cell's production of antiviral proteins.

The objective of this project is to investigate if epigenetic modulations could be associated with influenza virus infection using H1N1 and H5N1 viruses. This may lead to finding markers for innate and acquired protection to infection, which can be used to combat the virus.

Methodology

Investigation of Epigenetic markers both in vitro and in vivo models.

- *IN VITRO*
  - How epigenetic phenomena interfere with the virus pathogenesis.
  - Detection of global epigenetic marks (Histon modifications and DNA methylation
  - ChipSeq analysis of key genes
  - Proving the consept by the use of transgenic/knockout cells for epigenetic enzymes.
- *IN VİVO*
  - epigenetic marks on the acquired protective immunity in mouse infection models
- IN VİVO vs IN SILICO
  - Epigenetic status of the immune system modulation
    - DNA microarray hybridizations
    - chromatin immunoprecipitations
    - mass spectrometry analyses

Research questions to be answered !!!

- Does an influenza virus infectomics retain, acquire or modulate epigenetic markers?
- How does the epigenetic status effect the host cell sensitivity to infection?
- Are there certain epigenetic modifications induced by infection which are essential to virus replication?
  - Do these modifications reveal protection markers which can mitigate with infection?

What is new! answered !!!

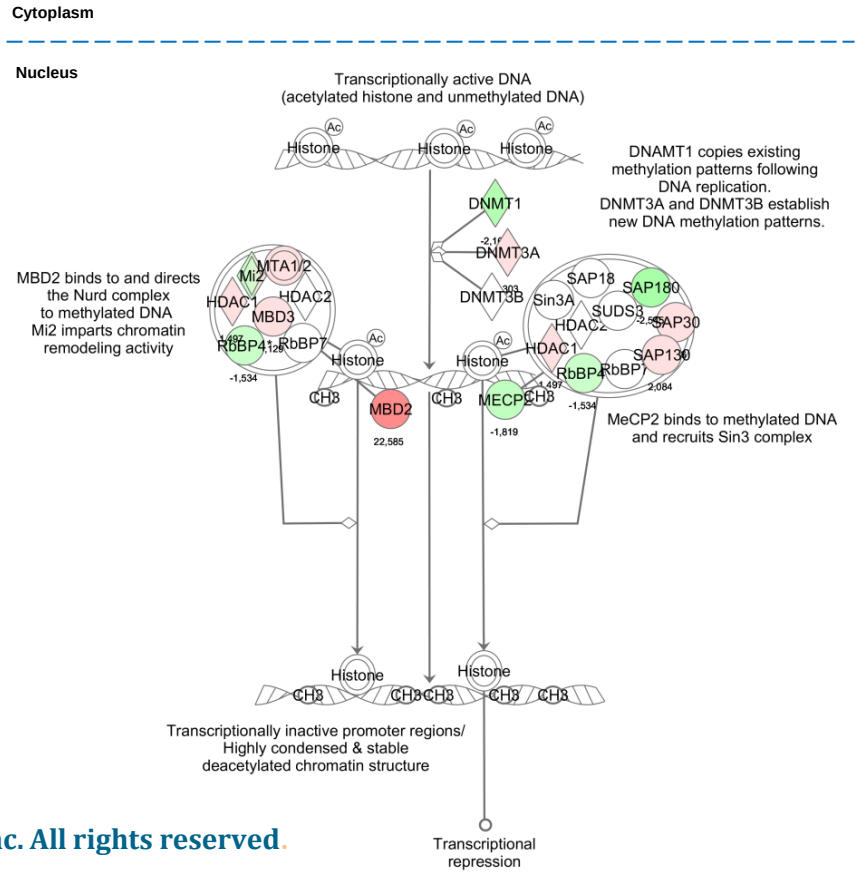
in vitro! in silico! in vivo !

***In silico* modelling of DNA methylation and transcriptional repression signaling in H1N1 *in vitro* infection.**

Ingenuity Pathway Analysis (IPA) demonstrates regulation of DNA methylation pathway during *in vivo* IAV infection.

Genes depicted in red are increased and genes in green are decreased compared to mock-infected mice.

Colour intensity increases with the magnitude of fold-change.



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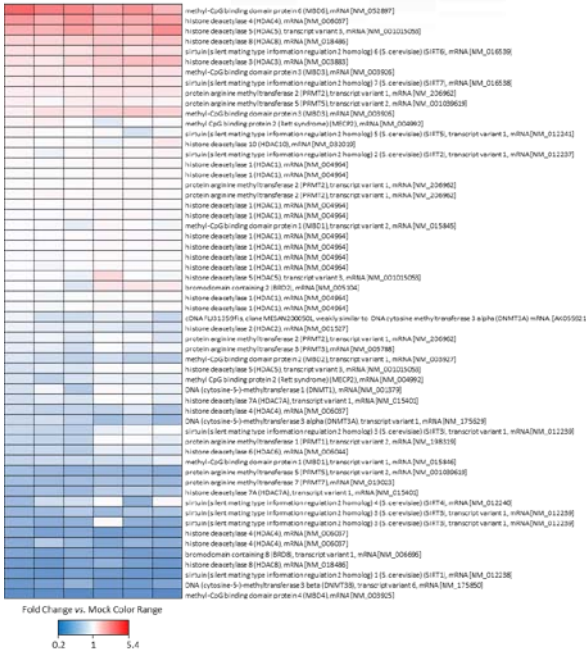
in vitro screening

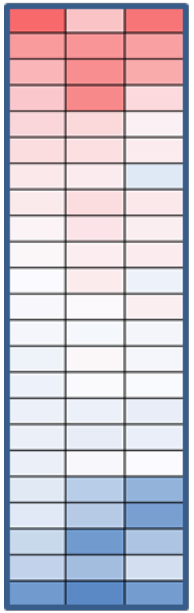
Agilent Microarray Platform
Analysis of Histon Modificaitons
Human pulmonary cells infected ith H1N1
1 day post infection – MOI=5

Log scale fold change of virus infected versus mock infeted cells.



in vivo screening

Agilent Microarray Platform
Analysis of DNA methyl-transferase
Mice lungs
3 days post infection – 1.10^5 PFU/mouse

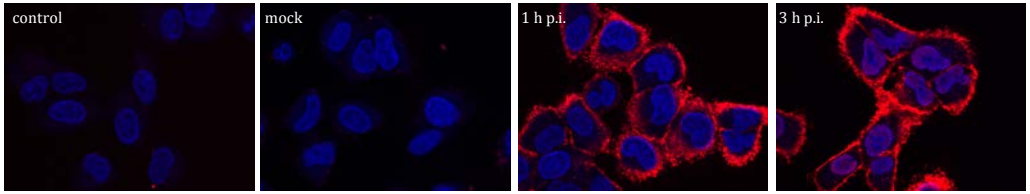


Gene Symbol	Genbank Accession
LOC668932	XM_001004878
Dnmt3a	NM_007872
Dnmt3l	NM_019448
Mgmt	AK090389
Dnmt3a	NM_007872
AK089219	AK089219
2700097O09Rik	AK079348
Dmap1	NM_023178
Dnmt1	NM_010066
N6amt1	NM_026366
Mtr	AK037599
Prmt6	AK087608
Mettl5	BC027547
N6amt2	NM_026526
Dmap1	NM_023178
Dnmt3a	NM_007872
Dnmt3b	NM_010068
Thumpd2	AK012806
Rnmt	AK031780
AK138093	AK138093
2700097O09Rik	AK079348
Mgmt	AK090389
Mettl7a	AK008925

What is new! answered !!!

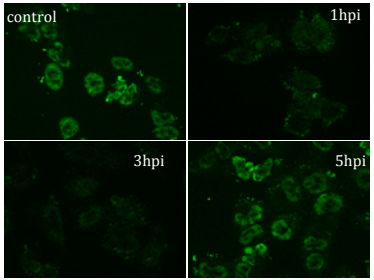
What is new! / answered !!!

H1N1 virus infection in pulmonary cells

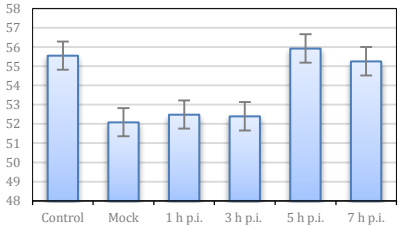


DNA Methylation

DNA methylation (5meC antibody)



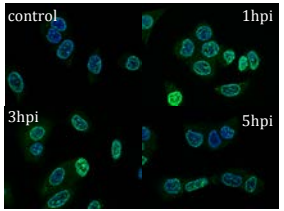
LUMA (LUminometric Methylation Assay)
A high throughput method



Global transient demethylation is observed rapidly in early infection period

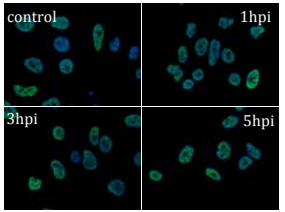
Histon modifications

H3K9 trimethylation
(repressive mark)



Drastic changes are observed at 1 and 3hpi (intensity/distribution of the signal) , Rehearses back to the control level progressively from 3 to 5/7hpi

H4K20 trimethylation
(another repressive mark)



No changes were observed upon viral infection

Our genetic code is not Destiny !
The discoveries in epigenetics may rewrite the rules of disease, heredity, and identity

FINANCIAL SUPPORT: