the open-source outbreak: H1N1, the olympics and new directions for public health

dr. jennifer gardy
bc centre for disease control
genome research laboratory

H1N1
outline

pandemic H1N1: the first open-source outbreak

H1N1/Olympics research project

descriptive epidemiology

predictive epidemiology
genomics enables:

present: descriptive epidemiology of a bacterial/viral pathogen

future: predictive epidemiology via genome surveillance
part 1

pandemic H1N1: the first open-source outbreak

H1N1/Olympics research project

descriptive epidemiology

descriptive epidemiology
rewind to march 2009
increased flu activity in Mexico
open source outbreak
sharing germs,
sharing genomes
increased flu activity in Mexico

april 25: 1st genome

CDC

California

WHO

APRIL 2009

pandemic!
April 26: International wiki
13 people, 8 institutes, 4 countries
http://tree.bio.ed.ac.uk/groups/influenza
Phylogenetic position of A/California/04/2009 for each genomic segment.
Analysis by Andrew Rambaut 26 Apr 2009
Preliminary Neighbor-Joining trees using the HKY distance metric.

Blue and green shading denote clades of related strains although extensive reassortment and heterogeneous sampling means that these clades contain different sets of strains.

See below for PDF versions of all these trees.

april 26: origins of the virus calculated
Rapid communications

The origin of the recent swine influenza A(H1N1) virus infecting humans

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5 days from sequence to open-access paper
increased flu activity in Mexico

april 25: 1st genome

may 6: 69 virus’ RNA

pandemic!
virus entered human population late 08/early 09
Pandemic Potential of a Strain of Influenza A (H1N1): Early Findings

Christophe Fraser,¹* Christl A. Donnelly,¹* Simon Cauchemez,¹ William P. Hanage,¹ Maria D. Van Kerkhove,¹ T. Déirdre Hollingsworth,¹ Jamie Griffin,¹ Rebecca F. Baggaley,¹ Helen E. Jenkins,¹ Emily J. Lyons,¹ Thibaut Jombart,¹ Wes R. Hinsley,¹ Nicholas C. Grassly,¹ Francois Balloux,¹ Azra C. Ghani,¹ Neil M. Ferguson¹†;

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increased flu activity in Mexico

April 25: 1st genome

May 6: 69 virus’ RNA

June 11: 250+ papers

Pandemic!
SARS, 2003

- *day 0*: virus isolation
- *day 19*: one viral genome

H1N1, 2009

- *day 0*: virus isolation
- *day 19*: 100+ viral genomes
- *where/when it arose*: multiple papers
- *vaccine seed strain*
how?
technological advances
shift in scientists’ attitudes
genomes = easy, cheap, fast
human genome project (1990)
10 years to draft
3 more to complete
$3 billion
100s of people
spring 2009
four weeks
$48,000 worth of reagents
three-person team
data = easy, cheap, fast
the file-sharing generation
Open Access Week - October 19-23, 2009
To broaden awareness and understanding of Open Access
openaccessweek.org
PLOS PUBLIC LIBRARY of SCIENCE
Biomed Central
The Open Access Publisher
PubMed Central
An archive of biomedical and life sciences journal literature
85% of scientists support open access

Mann et al., Comm. of the ACM 52(3):135. (2009)
collaboration
believed to be the same as his 'Fundstellen', types (a) and (b) being the most conspicuous.

The type IV alveoli (Fig. 1A and B) are found in adult males only, scattered amongst type II. They are composed of a number of small cells, type (g), which became filled with purple-staining granules after the last menses ceased.

A more detailed description of the salivary alveoli and of the changes which they undergo during the life-cycle of the tick will be published at a later date.

I am indebted to Mr. M. Urich of the Photographic Department, South African Institute for Medical Research, for the photomicrographs.

Department of Entomology
South African Institute of Medical Research
Johannesburg
June 22.

W. M. Till

BACTERIOLOGY
Bacteriophage Typing Applied to Strains of Brucella Organisms

Surface antigens usually limited to our taxonomy have been determined through the bacteriophage sensitivity of bacterial species. By such methods, the specificity of Brucella can be distinguished and the degree of sensitivity is used for typing strains of B. abortus 1:3 and strains of other bacteria.

Brucella phages were discovered only after rigorous attempts to identify them, and are not yet described in detail. A Brucella phage grown on strain 19 Brucella abortus in a flask culture has now been found to be active on cultures of B. abortus laboratory strains, but not on Br. melitensis and Br. suis. This phage was obtained by growing a single plaque taken from the end point dilution of a plaque suspension kindly supplied by Dr. W. A. Stobbs from the University of Leeds.

The phage produces irregular plaques of small diameter, which are not recognized as phages in the bacterial host on 'Albini' agar. These spots and the age of the phage appear to be strongly influenced by the host bacteria, and the incubation time at 37°C before these plaques appeared on the surface of the plate.

The technique found most practical is as follows: A 27-hr, aerated liquid culture of phage is prepared by centrifugation at 3,000 rpm for 20 min. and the supernatant heated at 60°C for 50 min. to destroy any remaining bacteria. The phage suspension is stored at 4°C and serially diluted tenfold before use.

The phage dilutions are spotted on dry 'Albini' agar plates by means of a 0.1 ml, diameter platinum loop. After drying the spots are covered with a suspension of young cells taken from a sterile culture and incubated at 37°C in an atmosphere of 5% carbon dioxide and 95% nitrogen, 72 hr.

Induction of Phage Formation in the Lyogenic Escherichia coli K-12 by Mitomycin C

Mitomycin C, a newly isolated antibiotic, is an effective inducer of spontaneous mutations (10). Its mechanism of action has been the subject of recent studies (10). It has also been observed that the induced deoxyribonucleic acid (DNA) damage in bacteria results from the action of mitomycin C (10). This damage is thought to be the result of DNA strand breaks induced by DNA polymerase (10).

In E. coli K-12 induced by mitomycin C, DNA polymerase activity and DNA strand breaks are found. The extent of DNA strand breaks correlates with the amount of DNA polymerase activity (10).

A new method has been developed for the determination of DNA polymerase activity in E. coli K-12. This method is based on the measurement of DNA synthesis in the presence of various concentrations of mitomycin C. The DNA synthesis is measured by the incorporation of radioactive thymidine into DNA and the uptake of radioactive thymidine into DNA is measured by the incorporation of radioactive thymidine into DNA (10).

Takyrase inhibition stabilizes axin and antagonizes Wnt signalling

Shih-Min A. Huang, Yuj M. Mishina, Sharmila Liu, Atwood Cheng, Frank Stegemeyer, Gregory A. Michaud, Olga Chariot, Elizabeth Welleit, Yue Zhang, Stephanie Wissinger, Marc Hilf, Xiaoyi Shi, Christopher J. Wilson, Craig MacKinnon, Victor McKeon, Aleem Fazal, Konrad Tomlinson, Fabrizio Serafin, Wenlin Shao, Hong Cheng, Michael Schütz, Cristina Rau, Markus Schütze, Judith Schlegel, Sonja Gfeller, Stephen Fawell, Chris Liu, Daniel Curtis, Marc W. Kirschner, Christoph Lentner, Peter M. Finas, John A. Tallarico, Tewis Bouwmeester, Jeffery A. Porter, Andreas Bauer, T. Feng Cong

The stability of the Wnt pathway transcription factor β-catenin is tightly regulated by the multi-subunit destruction complex. Disregulated Wnt pathway activity has been implicated in many cancers, making this pathway an attractive target for anticancer therapies. However, the development of targeted Wnt pathway inhibitors has been hampered by the limited number of pathway components that are amenable to small molecule inhibition. Here, we used a chemical genetic screen to identify a small molecule, XAV939, which selectively inhibits β-catenin-mediated transcription.

Using a quantitative chemical proteomic approach, we discovered that XAV939 stabilizes axin by inhibiting the poly-ADP-ribosylating enzymes tankyrase 1 and tankyrase 2. Both tankyrase isoforms interact with a highly conserved domain of axin and stimulate its degradation through the ubiquitin-proteasome pathway. Thus, our study provides new mechanistic insights into the regulation of axin protein homeostasis and presents new avenues for targeted Wnt pathway therapies.

This effect is mediated by the inhibition of β-catenin degradation by stabilizing axin, the concentration-limiting component of the destruction complex. Using a quantitative chemical proteomic approach, we discovered that XAV939 stabilizes axin by inhibiting the poly-ADP-ribosylating enzymes tankyrase 1 and tankyrase 2. Both tankyrase isoforms interact with a highly conserved domain of axin and stimulate its degradation through the ubiquitin-proteasome pathway. Thus, our study provides new mechanistic insights into the regulation of axin protein homeostasis and presents new avenues for targeted Wnt pathway therapies.

XAV939 inhibits Wnt signalling by increasing axin levels

XAV939 was identified as a small molecule inhibitor of the Wnt/β-catenin pathway from a high-throughput screening using a Wnt/β-catenin TopFlash (Wnt) reporter assay in HEK293 cells (Fig. 6a). XAV939 strongly inhibited Wnt3a-stimulated STF activity in HEK293 cells, but did not affect CSE, NRE or Wnt3a-stimulated STF reporter activity in C2C12 cells (Fig. 6b). In contrast, 100 nM XAV939, a close structural analogue of XAV939 (Fig. 6a), had no effect on the Wnt3a-induced STF reporter activity (Fig. 6b). XAV939 treatment blocked Wnt3a-induced accumulation of β-catenin in HEK293 cells (Fig. 6c), indicating that the compound modulates Wnt signalling upstream of β-catenin. Interestingly, XAV939 also inhibited STF activity in SW480 cells, a colorectal cancer cell line harbouring a truncated APC (Fig. 6d). XAV939 decreased β-catenin abundance, but significantly increased β-catenin phosphorylation (Fig. 6e), indicating that XAV939 promotes the phosphorylation-dependent degradation of β-catenin by inhibiting the activity of the degradation complex.

To explore how XAV939 may increase the activity of the destruction complex, we investigated whether compound treatment alters the protein levels of known Wnt pathway components. Notably, the
pandemic H1N1: the first open-source outbreak

new model for rapid response

fast, open genomic data

collaborative process

open access to results
part 2

- pandemic H1N1: the first open-source outbreak

- H1N1/Olympics research project

- descriptive epidemiology

- predictive epidemiology
surveillance, detection, diagnosis, treatment, & prevention of IDs

Divisions

The day to day public health work of the BC Centre for Disease Control is done in support of regional health authorities, the BC Ministry of Health and the Provincial Health Officer. Scientific and technical support is provided by the following specialized, yet integrated, operating divisions:

- Epidemiology Services
- Vaccine and Pharmacy Services
- Hepatitis Services
- STI/HIV Prevention and Control
- Tuberculosis Control
- Mathematical Modeling Unit
- Public Health and Emergency Management
- Environmental Health Services
- UBC Centre for Disease Control

Formerly BCCDC Laboratory Services, the BCCDC Public Health & Microbiology Reference Laboratory is a service of PHSA Laboratories.
BCCDC & pH1N1: lab testing

- April/May: surge in lab test volume
Virus Detections and Percentage of Respiratory Specimens Submitted to BC Provincial Laboratory Diagnosed Positive for Influenza Virus, per Week, BC, 2008-2009
BCCDC & pH1N1: research & activities

• “one-stop pandemic shop”
• sero-epi survey
• vaccine uptake campaign
• mathematical modelling
• informatics infrastructure
• genomics

[Logos: Genome British Columbia, BC Centre for Disease Control]
sequence 400-500 H1N1 genomes, observe viral evolution in real-time.
“influenza virus is sloppy, capricious and promiscuous” – world health organization
H1N1 genomics project overview

**Influenza testing by qRT-PCR (PHSA Labs)**

- Influenza A positive
- Influenza A/B negative

**Clinical Testing at BCCDC**

**Proposed Research**

Detect key mutations (20-60/d)

N=4800

- Drug-resistant
- Re-assortants and mutants
- Outbreak
- Typical SIV
- Severe disease

**Rapid sequencing of key regions (2-4 samples/d)**

N=500

**Virochip and Luminex (RVP)**

(BCCDC)

Mid disease
Severe disease
Unknown

Virochip

50

Luminex

50

Unknown

32

**Whole genome sequencing (20-40 samples/14d)**

(RTPCR @ PHSA Labs/BCCDC)

 sequencing @ BCGSC

N=500

**Chaperonin-60 sequencing**

(U. Sask.)
1. targeted sequencing: public health outcomes

- SNP-type and sequence key regions
- monitor changes
- adjust public health interventions as needed
- identify interesting virus

- e.g. 4 samples with point mutations in M gene rendered typing assay probe ineffective = new probe.
2. whole-genome sequencing: evolution

- monitor viral evolution in real-time
- determine effect of Olympics on viral evolution

Whole genome sequencing (20-40 samples/14d) (RTPCR @ PHSA Labs/BCCDC) (sequencing @ BCGSC) N=500

Virochip and Luminex (RVP) (BCCDC)

Chaperonin-60 sequencing (U. Sask.)
3. metagenomics: co-infections

- capitalize on available samples
- explore patterns of co-infection
BCCDC’s H1N1 genomics project

- all sequence will be made publicly available
- collaborating with social scientists, FNIH, GSC, international group of phylodynamics researchers using orwik, GoogleWave

vancouver2010.com
part 3

- pandemic H1N1: the first open-source outbreak
- H1N1/Olympics research project
- descriptive epidemiology
- predictive epidemiology
what is descriptive epidemiology of a pathogen?

where did it come from, how is it spreading, what makes it pathogenic?
story 1: where did it come from? SARS

- first novel EID of 21st century
  - Nov. 2002 – atypical pneumonia, China
  - March 2003 – international spread
  - July 2003 – containment (~800 deaths)

- suspected animal origin

- sequenced by BCCDC & others
but... high nucleic acid identity, not found in wild civets
• SARS CoV and others found in bats
• older, evolutionarily stable
• endemic since mid-1980s
story 2: how is it spreading? influenza

- IGSP: 4000 influenza genomes across time, space, species, type
source-sink model of emergence
co-circulating lineages w/ reassortment

doi:10.1371/journal.ppat.1000133
antiviral resistance is dynamic
story 3: what makes it pathogenic?  

Dengue

- 50-100 million infections per year
- four serotypes, each with multiple genotypes, geographic distribution
- large-scale sequencing effort underway (target= 3500 genomes)
- genomic correlates of severity
• DENV-2 SE Asian genotype replaced DENV-2 American

• mutations in E genes (receptor interaction), NS1, NS5, multiple UTRs (translation, replication)

• human genetic correlates: HLA (susceptibility), SNPs (severity)
bccdc story 1: outbreak evolution

• 36 complete *M. tuberculosis* genomes from VI outbreak to compare molecular evolution vs. field epidemiology data
bccdc story 1: unusual isolate *S. pneumo*

- genome from serotype 5 DTES outbreak contains an unusual genomic island (sugar usage?)
descriptive epidemiology: the future

• can answer questions around origins, evolution, pathogenicity, but not clinically-relevant questions

• effect of co-infections? links between evolution of co-infecting pathogens? role of immunity? viral quasispecies within an individual? epistatic interactions? genomes of most common pathogens? virus discovery?

• genome data must be collected along with extensive host, co-infection data
Recommended essential minimum data for SARI surveillance

General information
- Unique identification number
- Medical record number
- Name (of patient and parent’s name, if a minor)
- Date of birth
- Sex
- Address
- Date of onset of symptoms
- Date of collection of epidemiologic data
- Suspected novel influenza case
- Inpatient or outpatient

Clinical signs and symptoms
- Fever >38°C
- Cough
- Sore throat
- Shortness of breath/difficulty breathing
- Other clinical danger signs (19,22,23)

Type of specimen collected and date of collection
- Throat swab specimen, date of collection
- Nasal swab specimen, date of collection
- Other specimen (if collected), date of collection

Preexisting medical conditions
- Liver disease
- Kidney disease
- AIDS, cancer, or other immunocompromised state
- Neuromuscular dysfunction
- Diabetes
- Heart disease
- Lung disease
- Smoking history

Optional data collection for SARI surveillance

General information
- Diarrhea
- Encephalopathy

Exposure
- Occupation of patient
- Part of an outbreak investigation
- Contact with sick or dead poultry or wild birds
- Contact with friend or family who has SARI
- Travel in an area known to have endemic circulation of avian influenza (H5N1)
- Other high-risk exposure (e.g., eating raw or undercooked poultry products in an area of influenza virus [H5N1] circulation)

Vaccine/treatment history
- Vaccination against influenza within the past year
- Currently taking antiviral medicine

* SARI, severe acute respiratory infection; ILI, influenza-like illness.

Strategy to Enhance Influenza Surveillance Worldwide

Justin R. Ortiz, Viviana Sotomayor, Osvaldo C. Uez, Otavio Oliva, Deborah Bettels, Margaret McCarron, Joseph S. Bresee, and Anthony W. Mounts
genomics has been useful for many aspects of DE, but more is needed

CDC-like centres ideally positioned to lead/participate in future DE projects
part 4

pandemic H1N1: the first open-source outbreak

H1N1/Olympics research project

descriptive epidemiology

predictive epidemiology
stopping the next outbreak before it starts
months of undiscovered circulation in people

sometimes cover-ups, infrastructure problems
most often poor surveillance, novel pathogens
genome surveillance
population sampling to pick up threats before the lab or clinic
predictive epidemiology: genome surveillance

- genomics technology exists, global sentinel system is the roadblock
- needs infrastructure, standards, reporting
- local/national sentinel systems effective, start by incorporating genomics into these
- must ultimately consider diverse species, geography, demographics over time to be effective
sewagenomics
the end

thank you: patrick tang and bob brunham at bccdc, our local collaborators at the GSC and brinkman labs, and genome bc for their generous support.