Microarrays for Viral Pathogen Detection and Discovery

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March 26, 2006
Summary of Presentation

• Using the Virochip for Pathogen Detection and Discovery
• E-Predict: A Computational Tool for Virus Prediction
• Hospitalizations from Critical Illness
  – Case Report: a 28 year-old young adult female with fever and hypoxia
Why Pathogen Discovery?

- Many common infectious diseases have unrecognized viral causes (i.e. respiratory infections)
- Many diseases once thought to be noninfectious actually have a viral origin (i.e. cancer)

Pie chart showing:
- Human Papilloma Virus: 89% of Cervical Cancer
- Hepatitis B/C: 81% of Liver Cancers
- Helicobacter pylori: 56% of Stomach Cancers
- HIV/HHV-8
- Helminths
- EBV
- HTLV-1
Limitations of Current Detection Methodologies

- Viral culture – many viruses not culturable
- Immunoassays – require special reagents, candidate viruses
- PCR / RT-PCR – limited breadth (candidate viruses)
- Subtractive Hybridization – low throughput, laborious
- Expression cDNA-library screening – low sensitivity

Solution: Develop a comprehensive, unbiased, and high-throughput method to detect viruses from clinical samples
The Virochip

- A DNA microarray allowing simultaneous screening of all known viruses
- **Global** approach to detection
- Develop a comprehensive picture of which viruses are present and how frequently
The Virochip Pentathlon

Clinical Investigation

Genomics

Epidemiology

Bioinformatics

Virology

WOC, 2006
Selection of Viral Sequences
Example of Viral Genome Sequence Coverage (70mer oligonucleotides)
Isolate RNA and reverse transcribe into cDNA

Couple fluorescent dyes to cDNA

Mix fluorescent cDNA and hybridize to the microarray
Virus Chip Version 3

22,000+ viral sequences representing all viral species in GenBank as of June 2004.

(>1.5 Megabases of sequence)
“E-Predict”

Experimental Observations

- Environmental or clinical sample
- RNA/DNA
- Microarray
- Hybridization pattern

Probe selection

Pattern to profile comparisons

Predicted observations

- Environment
- GenBank
- Alignment to microarray probes
- Theoretical energy profiles

Urisman, 2005

virus_1
virus_2
virus_3
\ldots
virus_k

Ranked viral identities and probability estimates
Array: MegaViroP2-162:4258:Amplified RNA from Clinical Sample

<table>
<thead>
<tr>
<th>Iteration: 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family: Paramyxoviridae</td>
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<tr>
<td>11234  Measles virus</td>
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<tr>
<td>9626945_519_rc Measles virus</td>
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<tr>
<td>9626945_472_rc Measles virus</td>
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<tr>
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<tr>
<td>9630645_416  Canine distemper virus</td>
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<tr>
<td>9630645_441_rc Canine distemper virus</td>
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</table>

<table>
<thead>
<tr>
<th>E-Predict: A sample from the clinic</th>
</tr>
</thead>
</table>
### Array: MegaViroP1-256:2663:SARS_7

#### Iteration: 1

**Family: Coronaviridae**

<table>
<thead>
<tr>
<th>Accession</th>
<th>Description</th>
<th>Genus</th>
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<tr>
<td>9626535.1099</td>
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<td>9635572.255</td>
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<tr>
<td>15081544.766</td>
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<tr>
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<td>Bovine coronavirus</td>
<td>Coronavirus</td>
<td>Coronaviridae</td>
<td>0.371530</td>
</tr>
</tbody>
</table>

**Family: Astroviridae**

<table>
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<th>Accession</th>
<th>Description</th>
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<tr>
<td>219688</td>
<td>Mink astrovirus</td>
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<tr>
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<td>9630726.269</td>
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<td>21335364.350</td>
<td>Equine rhinitis B virus</td>
<td>Picornaviridae</td>
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Which Diseases to Target with the Microarray?

Six Key Selection Criteria:

- defined clinical entity
- epidemiological evidence for an infectious etiology
- specific target group
- focal pathology (e.g. inflammation)
- sample availability
- clinical relevance
Current Virochip Projects
Hospitalizations from Critical Illnesses
The United States CDC UNEX Model

• Population-based surveillance for unexplained death and critical illness possibly due to infectious causes (UNEX) in previously healthy young individuals (Hajjeh, 2002)
• 62% of cases fatal, 79% autopsies
• Infectious agent identified for only 34 / 122 cases (28%)

Goal – to use the Virochip as a comprehensive screening tool to better identify infectious agents in such cases
Case Report: a 28 year old healthy adult with fever and shortness of breath

History of present illness:
- 10 day history of fever, cough, night sweats, bloody sputum, and muscle pain
- Evaluated 3 days prior and treated empirically as an outpatient with oral azithromycin

Presentation:
- Fever
- Hypoxia
- Elevated white blood cell count
Chest CT Scan upon Admit, Representative Slice
Treatment:
- Patient was treated with ceftriaxone and doxycycline.
- On hospital day 3, the patient progressed to acute respiratory failure.
- Antimicrobials were changed to moxifloxacin and oseltamivir; high-dose methylprednisolone was initiated.

On hospital day 6, an open lung biopsy was performed:

Organizing bronchiolitis but no viral inclusions, multinucleated giant cells, or vasculitis.
**Diagnostic Tests:**
- blood, urine, and sputum viral, bacterial, and fungal cultures
- urine *Legionella* antigen
- Serum rheumatoid factor and anti-nuclear antibody
- Serum *Cryptococcal* antigen
- Coccidioidomycosis, histoplasmosis, *Mycoplasma, Chlamydia* Ab titers
- HIV serum antibody
- *Bordetella pertussis* DFA and PCR
- Immunofluorescence test for *Pneumocystis jiroveci*
- Serology tests for blastomycosis, tularemia, sporotrichosis, Q fever, and leptospirosis

**Viral Assays:**
- shell vial assay for cytomegalovirus
- DFA tests for respiratory syncytial virus, adenovirus, influenza A/B, parainfluenza virus types 1, 2, and 3
- Metapneumovirus PCR
- SARS coronavirus PCR
- ELISA for hantavirus (Sin Nombre)

All tests returned negative!

**ViroChip Analysis:**
Endotracheal aspirate from hospital day 8.
**Human Parainfluenza Type 4**

**ViroChip Result:**
Parainfluenza-4 (HPIV-4)

**Confirmation by specific PCR:**

![Diagram showing the genomic organization of HPIV-4 with markers and percentages representing similarity to other strains.](Diagram.png)
Human Parainfluenza Type 4

ViroChip Result:
Parainfluenza-4 (HPIV-4)

Confirmation by serology:

IFA with hospital day 21 sera (1:512)
Parainfluenza Virus

- ssRNA virus
- 4 serotypes
  - **Type 1**: Frequent cause of croup (laryngotracheo-bronchitis) in children
  - **Type 2**: Similar to Type 1 but less severe disease
  - **Type 3**: Important cause of bronchiolitis and pneumonia
  - **Type 4**: Generally mild disease

(Linda Stannard, University of Cape Town, S.A.)

Virus DFA Kits

Standard kits do NOT screen for PIV-4!
Acknowledgements

DeRisi Virochip Group
Joseph DeRisi
Don Ganem

Anatoly Urisman
Amy Kistler
Kael Fischer
Nicole Fischer
Patrick Tang
Silvi Reich

Hospitalization from Critical Illness
Bruce Patterson, Stanford
Jason Derek Merker, Stanford
Viral Discovery Follow-Up

**Phase I – Short-term:**
- Clone the genome from multiple samples / patients
- Recover or generate infectious clones
- Generate PCR-based diagnostic for rapid screening
- Develop probes for *in situ* hybridization assays
- Acquire more samples from another source

**Phase II – Long-term (linking the virus to the disease):**
- Develop immunoassay for rapid tests
- Expand screening to larger population
- Cell culture system for viral study
- Characterize individual proteins
- Establish an animal model