Genomics provides powerful tools in the global effort to combat Severe Acute Respiratory Syndrome (SARS)

Angel Tam

(Biochem 118Q)
Spring 2003
Professor Doug Brutlag

Angel Tam

Genomics provides powerful tools in the global effort to combat Severe Acute Respiratory Syndrome (SARS)

> Hundreds of stalwart young men in the uniform of their country [come] into the wards of the hospital in groups of ten or more. They are placed on the cots until every bed is full yet others crowd in. Their faces soon wear a bluish cast; a distressing cough brings up blood stained sputum. In the morning the dead bodies were stacked about the morgue like cordwood.

Colonel Victor C. Vaughan described this scene during his visit to Fort Devens, Massachusetts in September, 1918 (Kolata). The young men were victims of the Spanish Influenza, a devastating pandemic that infected 20 to 40 percent of the global population and killed over 20 million people in 1918 (National Vaccine Program Office). The Spanish Influenza was frightening because of its unknown origin. In March of 2003, when a mysterious disease called Severe Acute Respiratory Syndrome (SARS) began to gain worldwide attention, many feared that an epidemic reminiscent of the Spanish Influenza would ensue. Like the Spanish Influenza, SARS caught the world by surprise.

SARS was first discovered in Hong Kong, Canada, and Vietnam in early March, 2003. Patients had flu-like symptoms such as fever and malaise, which was followed by a nonproductive cough and shortness of breath. Eventually, some died because of progressive respiratory failure. By mid-April of 2003, there were over 4300 cases of SARS worldwide and 250 reported deaths in over 25 countries (Rota et al.). In response to the emerging disease, the World Health Organization (WHO) organized a global collaboration to identify the cause of SARS and to find cures for it. Progress came quickly. By the third week of March, 2003, labs in the United States, Canada, Germany and Hong Kong had identified a novel coronavirus from SARS patients. Within the next

month, this virus was completely sequenced. The genomic information proved to be quite powerful in the search for improved diagnostic tests and cures for SARS.

Discovery and sequencing of SARS-CoV

One of the first steps in fighting a new disease is to determine its cause. To see if SARS was caused by a bacteria, virus, or other agents, researchers obtained blood, sputum, and bronchial tissues from SARS patients and introduced them into cultures of Vero cells (monkey kidney cells). When these infected Vero cells started showing signs of death, they were observed under an electron microscope. This revealed black dots of genetic material inside spirally viruses, which clustered along the cell surface and at the endoplasmic reticulum (Grady and Altman). These images fit the description of a class of virus known as coronaviruses, which are known to cause approximately 20 to 30 percent of common colds in humans (Lovgren). Since the virus isolated from SARS patients was not identical to any known coronavirus, scientists gave it a new name: SARS-CoV.

It was surprising that a coronavirus was associated with SARS, because even though coronaviruses are known to cause serious diseases in animals, they are only known to cause mild colds and gastrointestinal problems in humans. Coronaviruses belong to family of enveloped viruses that replicate in the cytoplasm of animal host cells. They come in a wide variety, but are divided into three main groups: group 1 and 2 are the mammalian viruses, and group 3 is the avian viruses. All coronaviruses have genomes made of a single-strand plus-sense RNA approximately 30 kb long, which is the largest genome of any RNA virus (Rota et al.).

After SARS-CoV was isolated, the next step was to extract and sequence its RNA. On April 13, 2003, the Genome Science Center at the BC Cancer Agency in Vancouver successfully sequenced the entire genome of SARS-CoV from a sample taken from a SARS patient in Toronto (Marra et al). One day later, researchers at the Centers for Disease Control in Atlanta also completed their genetic sequencing, using a sample from a SARS victim in Hanoi, Vietnam. The sequences produced in the two studies differed by only 8 nucleotides (Holmes and Enjuanes). Since then, many labs have also sequenced strains of the virus (Figure 1). Analysis of the SARS-CoV genome revealed that it has all the common characteristics of a coronavirus, but it also has unique features that place it in a group separate from all previously known coronaviruses.

Knowing the genomic sequence of the SAR-CoV is crucial to understanding the molecular characteristics of the virus. Genomics provide a wealth of information that can aid in: 1) identifying the origin of the virus, 2) improving diagnostic tests, 3) studying the pathogenesis of the virus and developing treatments for the disease.

Tracing the origin of SARS

Data from the sequencing of SARS-CoV suggested that it probably did not result from a recombinant event between the known coronavirus strains. Other than one motif located in the 3´UTR (short untranslated region), there was also no indication of any exchange of genetic material between the SARS virus and non-coronaviruses (Marra et al.). Given the wide variety of coronaviruses found in humans, it was also possible that SARS-CoV evolved from a previously benign human coronavirus. But researchers later rejected this hypothesis, citing evidence that antibodies to SARS-CoV were absent in

those that were not infected with the virus, which would not have been the case had there been a closely-related predecessor in humans (Marra et al.).

In a review article in *Science*, leading coronavirologists Kathryn V. Holmes and Luis Enjuanes stated that it was unlikely that the SARS virus was genetically engineered, because at present times it would be impossible to modify 50% of the coronavirus genome without affecting its infectivity (Holmes and Enjuanes). This led them to believe that SARS-CoV had probably evolved from an animal virus that recently developed the ability to infect humans. Their hypothesis was correct: in May of 2003 scientists found a similar strain of the SARS-virus in civet cats in mainland China.

Improvement of diagnostic tests

To control the spread of the SARS virus, sensitive and specific diagnostic tests are needed to quickly identify potential SARS cases. Currently, diagnosis in the East and Southeast Asia regions is based on a clinical case definition that includes travel to specific locations or exposure to sick contacts (McIntosh). But using information now available about the SARS-CoV genome, scientists can deduce novel features that distinguish the virus from other circulating coronaviruses, which would help the development of PCR-based assays that specifically target the SARS virus (Marra et al.).

One advantage of using a PCR-based diagnostic test is that it is extremely quick; results can be read in just a few hours. It is also very sensitive, and like other viral tests, it becomes positive early in the course of the illness. The sensitivity of the PCR-based assay can not only allow for early detection of SARS cases, but any negative results may also help alleviate anxiety in the patients and community (McIntosh).. However, the technique is expensive and requires trained technologists. Thus, it may not serve as a

practical diagnostic tool until it is made readily and inexpensively available in local regions of outbreak.

Development of treatment for SARS

Genomics is not only useful in improving the diagnosis of a disease, it has therapeutic value as well. Ultimately, scientists hope to use their understanding of the SARS-CoV genome to develop vaccines and drugs to prevent and treat the disease. Because the sequence data of SARS-CoV from the various patients are quite similar, researchers believed that the virus is genetically stable in humans (Holmes and Enjuanes). This is good news because it means that the SARS virus does not have a high mutation rate, which makes the HIV virus so difficult to destroy.

A traditional way to deal with viral diseases is to develop vaccines that will prevent the virus from becoming active inside the body. The sequence of the SARS-CoV will help scientist to identify viral antigens that elicit immune response. This allows them to make genetically engineered viruses can express antigen proteins but not be transmitted from cell to cell (Holmes and Enjuanes), which will help in developing vaccines that confers immunity in local parts of the body.

However, given the global nature of SARS, it may not be practical to vaccinate everyone against the disease. A more modern approach is the use of antiviral drugs that combat viruses in infected individuals, just as antibiotics kill off bacteria. So far, there have been only about three dozen antiviral drugs on the market, and half of them were developed in the last 15 years to treat a single disease, AIDS. (Pollack) But prospects for making antivirals for SARS are looking well. Researchers have found that SARS-CoV encodes several proteins, one of which is 3CL protease (Holmes and Enjuanes). Like the

HIV protease, 3CL cleaves a polypeptide precursor encoded by SARS-CoV into several important viral proteins. Based on the sequence of the gene, scientists constructed a three-dimensional model of the protease, which will help in designing protease inhibitors that block replication of the coronavirus.

Other approaches to fighting SARS include passive immunization, which uses monoclonal antibodies to neutralize the virus. Recently, scientists experimented with a technique known as antisense. Antisense is aimed at disrupting the production of viral proteins. Complementary RNA strands, known as the "antisense," are made so that they would bind to viral messenger RNA and inhibit its translation into viral proteins. Although the premise of antisense is simple, its failures in treating diseases in the past have made some researchers skeptical. Thus, whether antisense can effectively treat SARS remains to be seen.

Although no effective cures have been found yet, the prospects of treating SARS is looking bright. The genomics of the SARS-CoV virus not only helped scientists to discover the origin of SARS, it also served as a crucial guide in the development of diagnostic tests and, ultimately, cures and vaccines for the disease. Ernesto Freire, a researcher at Johns Hopkins University, comments on the efforts thus far:

> It's been like a great detective novel, this race to find and stop the culprit behind this new disease. It's amazing how quickly we've made progress, from no one working on it two to three months ago, to having the bug identified and having its genome sequenced. This is the first epidemic of the 21st century, and the response from the public health community and the scientific community has been very good so far. (Purdy)

Figure 1: SARS Virus Strains Sequenced as of April 29 (Enserink and Vogel)

Institute	Strain
BCCA Genome Sciences Centre, Vancouver	Tor2 (complete)
Centers for Disease Control and Prevention, Atlanta	Urbani (complete)

University of Hong Kong	HKU-39849 (complete)
Chinese University of Hong Kong	CUHK-W1 (complete)
Genome Inst. of Singapore/Singapore General Hosp.	KYK (complete)
Academy of Military Medical Sciences/ Beijing Genomics Institute	BJ01, BJ02, BJ03, BJ04, GZ01 (partial)

Works Cited

Enserink, Martin and Vogel, Gretchen. "Hungry for Details, Scientists Zoom In on SARS Genomes," *Science.* **300**, 715 (2 May 2003).

Grady, Denise and Altman, Lawrence K. "Global Collaboration on SARS Bears Fruit," *The New York Times.* 26 May 2003, online edition.

Holmes, Kathryn V. and Enjuanes, Luis. "Perspective: The SARS Coronavirus: A Postgenomic Era." *Science.* **300,** 1377-1378 (30 May 2003).

Kolata, Gina. *Flu: The story of the great Influenza Pandemic of 1918 and the search for the virus that cased it.* New York: Farrar, Straus and Gircoux; 1999 (p. 16).

Lovgren, Stefan "Scientists Crack SARS Genetic Sequence," *National Geographic News*. 15 April 2003, online edition. http://news.nationalgeographic.com/news/2003/04/0415_030415_sarsdna.html

Marra et al. "The Genome Sequence of the SARS-Associated Coronavirus," *Science*. Published online 1 May 2003. http://www.sciencemag.org/cgi/content/full/300/5624/1399

McIntosh, Kenneth. "The SARS Coronavirus: Rapid Diagnostics in the Limelight," *Clinical* McIntosh *Chemistry.* **49**, 845-846. (2003). http://www.clinchem.org/cgi/content/full/49/6/845

National Vaccine Program Office, Center for Disease Control and Prevention. "Pandemic Influenza." http://www.cdc.gov/od/nvpo/pandemics/flu3.htm

Pollack, Andrew. "With SARS, Antivirus Arms Race Heats Up," *The New York Times.* 27 May 2003, online edition.

Purdy, Michael. EurekAlert! "Drug design expert sets his group's sights on SARS." Public release on 28 May 2003. http://www.eurekalert.org/pub_releases/2003 05/jhu-dde052803.php

Rota et al. "Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome," *Science*. Published online 1 May 2003. http://www.sciencemag.org/cgi/content/full/300/5624/1394