TECHNIQUES AND APPLICATIONS

Analysing influenza's sweet spot

A study published in the *Journal of Molecular Biology* describes an innovative new tool that can be used to monitor the potential threat posed to humans by viruses such as the H5N1 influenza virus. A team led by Ian Wilson and James Paulson at The Scripps Research Institute has used a carbohydrate (or glycan) microarray that allows detailed analysis of the receptor-binding specificities of influenza viruses.

The binding preferences of the major influenza viral coat protein, the haemagglutinin (HA), are an important property, as they determine whether influenza viruses can spread easily among humans. HA discriminates between glycan receptors present on the surface of human and avian cells. In particular, the way in which the sialic-acid monosaccharide links to the surface glycan molecule profoundly affects HA binding. HAs from human influenza viruses preferentially bind to so-called α2-6 receptors, which are plentiful on the surface of epithelial cells in the human respiratory tract. Once the virus replicates, shed virus can be passed in droplets that are spread by sneezing and coughing. Avian influenza viruses, including the H5N1 strains, bind mainly to α2-3 receptors, which are found on the surface of intestinal cells in birds. In humans, α2-3 receptors are found in the mucus glycoproteins that protect the cells in the upper airway. This binding preference is thought to restrict the spread of avian influenza virus in the human population.

Glycan microarrays use the same principles as DNA or RNA microarrays, but instead of nucleic acids, the slide is impregnated with sugars, each with different structures of interest. Using a glycan array that included the characteristic α2-3 and α2-6 receptors, the authors were able to detect fine differences in the binding specificities of a panel of recombinant HAs from H1 and H3 influenza viruses, including HAs from two strains that were circulating during the 1918 pandemic. As expected, HAs with the consensus avian sequence showed a preference for α2-3 glycans, whereas human HAs bound strongly to α2-6 glycans.

But analysis of the two 1918 pandemic strains revealed some intriguing differences. HAs from the South Carolina 1918 strain (18SC-KL) and the New York strain (18NY) differ by only a single amino acid (Gly225Asp) that is located within the HA-receptor-binding site. Although 18SC-KL showed a classic human binding profile, binding exclusively to α2-6 glycans, 18NY bound to both α2-6 and α2-3 glycans. The introduction of a single avian mutation into 18NY at position 190 (Asp190Glu, 18NY-Av) switched its binding profile to that of a classic avian HA. Structural models of the 18SC and 18NY HAs show that position 190 lies on top of the receptor-binding pocket. It seems that the Glu190Asp mutation increases the size of the binding pocket, which allows binding to α2-6 glycans.

The identification of the structural determinants of a major species barrier in 1918 H1 variants offers a foretaste of the important insights that can be gained by applying this technology to the analysis of emerging influenza viruses. Monitoring changes to the detailed 'receptor-binding footprint' generated by the microarray will be invaluable in the identification of emerging viruses that could cause new pandemics or epidemics.

Shannon Amoils

ORIGINAL RESEARCH PAPER Stevens, J. *et al.* Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. *J. Mol. Biol.* **355**, 1143–1155 (2006)

WEBSITES

Ian A. Wilson's laboratory:
http://www.scripps.edu/mb/wilson

James Paulson's laboratory:
http://www.scripps.edu/mb/paulson

Consortium for Functional Genomics:
http://www.functionalglycomics.org