Predicting Flu—Tracking the Evolution of Resistant Mutant Combinations

After earning his doctorate at the University of California, Los Angeles (UCLA), Dr Nicholas Ching Hai Wu (Croucher Post-Doctoral Fellowship 2015 - 2017) joined a research team at The Scripps Research Institute (TSRI) to track, characterize, and predict the evolution of the influenza virus. Wu has conducted research on HIV, molecular evolution, protein structure, and high-throughput methodologies as well.

In 1997, an instance of the bird flu made an unexpected jump from poultry to human, when a young boy died suddenly from severe flu symptoms after playing with chickens at a Hong Kong petting farm. Virologists were stunned, as no human, not even poultry workers, had contracted this particular strain of flu before. By year's end, this strain, designated as H5N1, spread to 18 people, killing six. The city's response was swift and draft—Hong Kong's health director ordered the immediate elimination of all poultry throughout the city.

Home from school due to city-wide closures, eight-year-old Nicholas Wu watched these events unfold with horror. Over a period of three days, one and a half million birds—chickens, partridge, geese, and quail—were hauled off for slaughter. Normally so much a part of Chinese culture, the absence of poultry trading and consumption put a damper on busy market places, holidays feasts, and reunions with family and friends. The familiar sounds of squawking poultry and bickering vendors at Wu's neighborhood poultry market evaporated overnight, overwhelming him with a sense of uneasiness and loss. "I was very surprised at how a virus can change the culture," Wu remembers.

But despite these drastic measures and the heightening of standards throughout the poultry industry, the bird flu returned to Hong Kong and other parts of Asia with a vengeance in 2003 and at periodic intervals since then. New mutations and strains have emerged, including the H7N9 strain in 2013, complicating the crisis even further.

Some twenty years later, Wu's boyhood trauma became his adult mission: to unfold the ages-old mystery of influenza—why it is so virulent and how it is able to evolve into so many distinct variations.

Each year, the World Health Organization develops a flu vaccine based on data gathered by scientists worldwide revealing new and prevalent flu strains. The vaccine works by generating antibodies within the circulatory system that gradually increase one's immunity against the flu.

In order to replicate, the flu virus must attach to special receptors located on the membrane that envelops human upper respiratory cells or red blood cells. A spiky protein called hemagglutinin seeks out these receptors, which are composed of sialic acid, a type of sugar. Each hemagglutinin spike scoops up a molecule of sialic acid and tucks it into a pocket located on the spike's tip known as the Receptor Binding Site (RBS). This process literally fuzes the virus to the receptors on the cell's membrane and allows the virus to penetrate the cell and begin propagating. The antibodies produced by a flu vaccination target the RBS to prevent the virus from entering the cell.

The reason influenza is able to evolve so quickly lies in its ability to generate numerous mutations—abrupt changes to the genetic information contained in the virus' DNA. Most of these mutations quickly kill off the flu virus but about one percent manage to penetrate the virus, altering its structure and allowing it to start propagating as a new viral strain.

Up until now, scientists have been in the position of "chasing" the flu—seeking out new strains and developing vaccines that build up immunity against those strains. Wu and his team wanted to take a more proactive approach: "If we know ahead of time what kind of mutations can be accommodated at a particular site on the virus, we'll have a better idea of how to develop drugs or antibodies to target that site." Wu says.

The experimental process was two-fold: Wu and his team needed to create all possible mutation for each viral strain, then test every combination of mutations possible to see which ones were virus-tolerant.

Using a genetic engineering technique known as saturation mutagenesis, Wu and his team introduced 30,000 mutant variants, each containing a single mutation, into the RBS of H1N1 and H3N2 flu strains. More than 95 percent of the variants died—not an unexpected result given that most single mutations tend to disrupt the RBS, killing the virus.

Wu then combined the same mutations into aggregates of twos and threes and repeated the process. A surprising 20 percent of the mutant combinations not only didn't kill the virus, they actually strengthened the viral strains, allowing them to keep replicating.

This discovery implied that certain mutations are able to bond together in predictable patterns to help the influenza virus thrive and propagate. Wu points out that the evolution of these resistant mutant combinations was not random—each new mutation developed in such a way as to cancel out the destructive effects of the previous one—a process known as epistasis.

"Epistasis refers to the combined effect of two individual mutations that can't be predicted ahead of time," Wu explains. "Individually, each of these mutations kills the virus, but together, they compensate for each other's harmful effects."

"This prevalence of epistasis fully demonstrates the complexity of influenza evolution," Wu continues.

Using a type of DNA sequencing technology known as next-generation sequencing (NGS), Wu and his colleagues identified each viable mutant combination and observed that many of them occurred in an area of the influenza RBS known as the 220-loop, a region already known for ease in facilitating the viral jump from birds to humans. What they didn't realize was just how tolerant the 220-loop would be when exposed to such a wide variety of mutant combinations. Contrary to the single mutations that interfered with the function of the RBS (similar to the way the vaccine antibodies function), certain mutant combinations did *not* disrupt the receptor binding function—a consideration that could impact the development and application of flu vaccination and influenza drug therapies in years to come.

"These results suggest that perhaps we should avoid targeting regions such as the 220-loop when developing antibodies or drug therapies since these regions appear to be relatively tolerant of mutations," Wu states.

Wu's research substantiates the epistatic effect in generating new flu strains. He and team are continuing to focus on generating new mutant combinations and analyzing which ones allow the

flu virus to pass through the cell membrane. “Preventing such strains from materializing in the first place would create a major breakthrough in the battle against influenza,” Wu says.

“Predicting the evolution of mutations, that is, knowing in advance what potential mutations will come up, could help us develop effective antibodies and target sites,” Wu continues. “For each combination of mutations we are able to predict and generate, we are one step closer to forecasting how flu evolves—potentially leading to its elimination entirely from the world stage.”

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