Receptor binding specificity of highly pathogenic avian influenza viruses: Development of improved assay system to determine the mutated H5 avian viruses that acquire human receptor specificity

Yasuo Suzuki[1,2] and Hiroaki Hiramatsu[1]
[1]*College of Life and Health Sciences and Health Science Hills, Chubu University*;
[2]*Global COE Program, School of Pharmaceutical Sciences, University of Shizuoka*

Influenza is one of the most broadly spread zoonotic infectious diseases in the world, and its pathogen, the influenza virus, is extremely mutable. Currently, highly pathogenic avian influenza (subtype H5N1), which is continuing to spread across Asia, Europe, and the African continent, has already been transmitted to humans and has been undergoing possible mutation for propagation among humans. When this new variant of the virus becomes an epidemic among humans, it will spread throughout the world within a short time resulting in a pandemic because humans do not have immunity to the virus.

Most avian influenza viruses bind preferentially to sialic acid (Sia)α2-3Galctose (Gal) (avian-type receptor), whereas human influenza viruses bind preferentially to Siaα2-6Gal (human-type receptor). Switch from Siaα2-3Gal to Siaα2-6Gal receptor specificity is a critical step in the adaptation of avian viruses to human hosts.

We (1-4) have detected that mutations of single or set of few amino acids at positions in the hemagglutinins (HAs) of H5N1 which are isolated in China, Vietnam and Thailand independently converted the virus known to recognize the avian receptor to ones that recognize the human receptor. Using the reverse genetics technique, we also identified the potential amino acids in HA of H5N1 which involved in the mutations responsible for the binding to human-type receptor. However, the assay system used in these reports takes relatively long time (for 2-3days) and needs high amount of viruses. In this report, we developed improved assay system to determine the mutated avian influenza viruses that acquire human receptor specificity.

This method included coating of synthetic *N*-acetylneuraminic acid α2-3 (or 6) Gal-glycopolymer synthesized from γ-polyglutamic acid, a component of traditional Japanese food, "Natto" (5) to the 96 well plate, virus binding to the polymer, fix the bound virus with formalin, trapping the virus with 1st antibody directed the virus, trapping the 1st antibody with 2nd antibody linked with the enzyme, and detection of the color developed with enzyme substrate. It takes for 6 hrs to determine the receptor binding specificity of the viruses that bind to avian- and human-type receptor with small amount of virus (8HAU) in culture fluid or allantoic fluid of virus-infected cells and embryonated chicken eggs, respectively. This method needs no expensive machine and may be useful to find the molecular signal of H5N1 avian viruses that acquire human-type receptor specificity and pandemic potential.

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