Glycoengineering for a Universal COVID-19 Vaccine

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The SARS-CoV2 surface spike protein is a rational target for COVID-19 vaccination. However, spike protein glycosylation helps the virus evade the immune system, undermining vaccination efforts against newer variants. Dr Chi-Huey Wong's group at Academia Sinica, Taiwan, is hoping to combat this by glycoengineering protein- and mRNA-based COVID-19 vaccines.

The Spike Protein Behind COVID-19 Infection

Since the emergence of COVID-19 in Wuhan, China, in 2019, the health burden has been huge, with over 750 million infections and 7 million deaths globally. Vaccination is regarded as the most effective intervention. Indeed, the rapid development of COVID-19 vaccines represents a triumph of scientific innovation over disease.

COVID-19 disease is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), an RNA coronavirus. COVID-19 vaccines target the virus's surface spike glycoprotein, known as S. This is made up of two regions, S1 (N-terminal side) and S2 (C-terminal side). S1 is composed of three main domains – an N-terminal domain, a receptor binding domain (RBD), and two subdomains (SD1/2). S2 is composed of five main domains – a fusion peptide proximal region, heptad repeat 1 (HR1), connecting domain (CD), heptad repeat 2 (HR2), and transmembrane domain. Three S proteins trimerise into a spike protein – S1 forms a bulb-shaped head, and S2 forms a stem that attaches to the viral envelope.

This structure is key to SARS-CoV-2's infectivity. The RBD region of S1 attaches to angiotensin-converting enzyme 2 (ACE2), a cell surface receptor. This enables SARS-CoV-2 virions to enter host cells. The virions hijack its cell's 'machinery' to replicate its genome, synthesise viral proteins, and assemble new virions. After escaping the host cell, the virions rapidly infect other cells. ACE2 is highly expressed in lung epithelial tissue, which is why COVID-19 infection manifests as respiratory complications.

The Importance of Glycosylation

Glycosylation – the attachment of sugar chains (glycans) to proteins – is an important post-translational modification. There are two ways that glycans attach to glycosylation sites (glycosites) – N-linked and O-linked. N-linked glycosylation involves the attachment of a glycan to an amide nitrogen of asparagine (Asn). Usually, the glycan is linked to Asn via N-Acetylglucosamine (GlcNAc). The presence of mannose moieties leads to branching.

There are three types of N-linked glycans – high-mannose, hybrid, and complex. High-mannose glycans contain terminal mannose moieties attached to a GlcNAc core. Hybrid glycans also contain a GlcNAc core and terminal mannose moieties but also contain further branches. Complex glycans contain a mannose and GlcNAc core and are elongated with repeated LacNAc units, often with a terminal sialic acid moiety (sialylation). O-linked glycosylation involves the attachment of a glycan to the oxygen of threonine (Thr) or serine (Ser).

The SARS-CoV-2 S protein is heavily glycosylated with host-made glycans. Mutations can lead to variability in glycan patterns – different 'glycoforms'. Emerging research indicates that spike glycans play a role in SARS-CoV-2's invasion of cells and evasion of the immune system. Dr Chi-Huey Wong leads a research group at the Genomics Research Center, Academia Sinica, Taiwan. The group explores the structural and functional implications of S glycans, and uses this knowledge to 'glycoengineer' more effective COVID-19 vaccines.

Investigating Glycosylation Patterns

The group has used a variety of in vitro cell lines to express S protein. These include human lung epithelial cells (BEAS-2B) and human embryonic kidney cells (HEK293T or HEK293S). This involves transfection of the cells with a vector containing a gene construct expressing the S protein. After cell culture, the expressed S is purified and undergoes a series of experiments – liquid chromatography-tandem mass spectrometry (LC-MS/MS) to assess glycoforms, ELISA and pseudovirus assays to assess human ACE2 (hACE2) binding, and negative staining electron microscopy (EM) to assess structure.

LC-MS/MS analysis revealed that compared with HEK293T cells, S protein expressed in lung cells had more complex glycans, with a greater diversity of processing – multiple antennae, galactosylation, fucosylation, and a higher degree of sialylation. In contrast, HEK293T-expressed S had more hybrid type and high-mannose glycans.

Investigating Spike Protein Binding to ACE2

How do glycans impact the binding of S to ACE2? The group conducted ELISA and pseudovirus assays to find out. ELISA studies showed that the more complex the S glycoform, the more strongly it bound to hACE2 (greater avidity). S glycoforms (such as those with only high-mannose glycans or with a single GlcNAc at each glycosite) bound to hACE2 with lower avidity compared to more complex glycoforms. Moreover, binding avidity was greater the more sialylated the S protein was. To confirm these findings, the group tested pseudovirus infection of hACE2-expressing HEK293T cells. Pseudoviruses are non-pathogenic artificial viruses that are unable to replicate.

These pseudovirus assays confirmed the importance of complex glycans and sialylation to SARS-CoV-2's infectivity of host cells.

The S protein has 24 glycosites – 22 N-linked and 2 O-linked amino acids. How would mutation of these glycosites affect infectivity? A panel of 24 pseudoviruses was generated, each with a single S protein mutation corresponding to each glycosite. N-linked Asn residues were substituted to glutamine (Gln), while O-linked Thr or Ser residues were substituted to alanine (Ala) to disrupt glycosylation. Five hACE2-expressing cell lines, including HEK293T, Vero-E6 (monkey kidney cells), and three human lung cell lines (A549, Calu-1, and Calu-3) were infected with these 24 pseudoviruses. Disruption of glycosylation was found to reduce infectivity. In particular, two N-glycosite mutations led to a complete lack of infectivity.

Glycan Shielding of Conserved Epitopes

From the GISAID (Global Initiative on Sharing Avian Influenza Data) database, the group extracted over 1 million S protein sequences for all available SARS-CoV-2 strains and conducted structural analyses. This led to a curious discovery – epitope regions that were highly conserved tended to be shielded by glycans, often linked to adjacent domains. Dr Wong hypothesised that exposing these conserved glycan-shielded regions could elicit immune responses against these conserved regions present in various variants; thus the vaccine becoming broadly protective.

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Developing a Glycoengineered Protein Vaccine

Based on these findings, the group developed a COVID-19 protein vaccine based on a low-sugar S immunogen. They expressed a high-mannose glycoform S protein trimer (SHM) in GnTI⁻ HEK293S cells. After purification, they treated the protein with endoglycosidase H, an enzyme that trims the glycans down to GlcNAc at each N-glycosite, producing a 'mono-GlcNAc–decorated' S trimer (SMG). As a comparator, they used a HEK293E-expressed fully glycosylated S (SFG) that contained diverse glycans, similar to current COVID-19 vaccines. Vaccine preparations were mixed with aluminium hydroxide adjuvant for immunisation.

The group immunised BALB/c mice, Syrian hamsters, and CAG-hACE2 mouse disease models, involving intramuscular injection with two doses of SMG, SHM, or SFG. The hamsters and CAG-hACE2 mice underwent viral challenge studies. These were immunised with SMG or SFG or control (phosphate-buffered saline) before being exposed to wild-type (WT) SARS-CoV-2. COVID-19 challenge studies were repeated in CAG-hACE2 mice but with Alpha, Gamma, and Delta SARS-CoV-2 variants.

The SMG vaccine elicited better humoral responses – including greater IgG titres and subtype diversity, as well as T-cell and B-cell responses. Challenge studies demonstrated superior protection against COVID-19 with SMG. SMG and SFG both led to reduced WT viral load and fewer legions in the lungs of hamsters and mice. SMG led to improved mouse survival outcomes versus SFG after exposure to all variants. A groundbreaking discovery was the isolation of an immune-boosting monoclonal antibody (mAb), m31A7, from SMG-immunised mouse B-cells; however, this antibody was not induced in SFG immunisation. Hydrogen-deuterium exchange mass spectrometry (HDX-MS) and cryo-electron microscopy (cryo-EM) studies showed that m31A7 interacts with a conserved epitope of the S protein's RBD – suggesting that it may prevent cell infection by blocking binding to ACE2.

From Protein to mRNA Vaccine Development

Following the development of the SMG protein vaccine, Dr Wong's group set their sights on developing a low-sugar mRNA vaccine. The global deployment of mRNA vaccine technology was first showcased during the COVID-19 pandemic. Unlike protein-based vaccines, mRNA vaccines provide a code, which is 'translated' into protein immunogens by cells' ribosomes. Various COVID-19 mRNA vaccines are approved, all encoding the S protein. However, a major challenge to effective vaccination is SARS-CoV-2 evolution. New variants may have spike protein mutations that could escape immune responses. The group discovered that of seven conserved epitopes in the S protein RBD, four had already mutated in the Omicron subvariant.

Identifying Conserved Epitopes

The group analysed over 14 million S protein sequences from GISAID with a mutation rate <1%, identified 17 conserved epitopes, and confirmed these using the Immune Epitope Database. Using protein structure simulation software, they determined that all but one of these epitopes were shielded by glycans, and that these are largely concentrated in the S2 stem region. Pairwise S sequence alignment with closely related coronaviruses revealed that SARS-CoV-2, SARS-CoV and MERS viruses share the same stem epitopes. Thus, the stem was a logical target in the group's mRNA vaccine development.

Developing a Glycoengineered mRNA Vaccine

The group used site-directed mutagenesis to generate de-glycosylated mRNA vaccines based on S proteins from Wuhan (WH) and Delta variants. This involved deletion of all N- and O-glycosites (deg-S), the deletion of all N-glycosites in the S2 region (deg-S2), or the deletion of six glycosites in the CD/HR2 (stem) region of S2. Unmodified glycosylated S mRNAs were used as a comparator. mRNAs were produced via in vitro transcription, purified, and encapsulated in lipid nanoparticles (LNPs).

After confirming translation in HEK293 cells, they conducted immunisation studies – BALB/c mice were injected intramuscularly with two doses of the mRNA-LNPs. Serum IgG ELISAs showed that S2 or stem deglycosylation elicited greater cross-reactivity against other SARS-CoV-2 variants and even against other coronaviruses, including HCoV-NL63, HCoV-229E, HCoV-HKU1, HCoV-OC43, MERS-CoV and SARS-CoV. Pseudovirus neutralisation assays showed that S2 deglycosylation led to 6- to 8-fold greater neutralisation activity against MERS-CoV and SARS-CoV. Moreover, deglycosylation led to stronger T-cell responses, demonstrated with splenocyte ELISpot assays.

The Way Forward

Dr Wong's research is driven by the urgent need for COVID-19 vaccines that are broadly protective against current and future variants. The rapid evolution of SARS-CoV-2 and spike protein mutations makes this challenging. Their strategy – 'de-shielding' glycans – has shown promising results for both protein and mRNA vaccine development. Remarkably, their mRNA vaccine demonstrated cross-reactivity not only against different variants but even MERS and SARS. This paves the way for a universal pan-coronavirus vaccine. For this to become a reality, these preclinical findings must be followed up with clinical trials and a phase 1 clinical trial is ongoing.

MEET THE RESEARCHER

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Dr Wong has been a Professor of Chemistry at The Scripps Research Institute since 1989. He is currently Scripps Family Chair Professor of Chemistry with a joint appointment as Distinguished Professor at Genomics Research Center, Academia Sinica. He also serves as President of IBMI (Institute for Biotechnology and Medicine Industry) and is a member of the Science and Technology Advisory Board of Executive Yuan, Taiwan. Dr. Wong obtained his PhD in Chemistry from the Massachusetts Institute of Technology in 1982 and then undertook postdoctoral training at Harvard University. His research focuses on developing new methods and tools for making and studying complex carbohydrates and glycoproteins, especially those associated with human diseases. Dr Wong has trained over 500 graduate students and postdoctoral fellows, published over 750 papers, and obtained over 120 patents. He served as President of Academia Sinica and advised various research, industry, and governmental stakeholders. He received worldwide recognition for his contributions to chemistry and human health, including the Wolf Prize, the Welch Award, and the Tetrahedron Prize, and is an elected member of the US National Academy of Sciences.

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FURTHER READING

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