Archives of Clinical Trials

Commentary

The Matryoshka code of COVID-19 mRNA vaccines: overlapping viral sequences?

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- Received Date: 01 Dec 2022
- Accepted Date: 05 Dec 2022
- Publication Date: 09 Dec 2022

We read with great interest the paper by Beaudoin CA et al. "Are There Hidden Genes in DNA/RNA Vaccines?", reporting overlapping sequences between the SARS-CoV-2 spike (S) glycoprotein and two viral genes [1]. If translated, the undesired proteins may cause rare, untoward effects, including those recorded in Vaccine Adverse Event Reporting System (VAERS).

These findings are in line with our own research and that of others however, aside from overlapping genes (OLGs), the S protein also contains overlapping molecular structures and signals (heptad repeats, simple sequence repeats, calcium calmodulin kinase II, and prion-like domains) that can lead to VAERS-recorded pathology [2-6].

Overlapping genetic and structural information are important to viruses, as they maximize the number of translated proteins derived from the same genetic information [7]. This compact arrangement also allows for the emergence of mutations without major genetic restructuring [8]. Furthermore, there is evidence that such structures also regulate gene expression in many viruses [9], including coronaviruses [10,11](Figure 1).

Taking the above viral-derived complication into account, messenger RNA (mRNA) vaccines encode the full-length S protein that when expressed on the surface of cells, prompts the generation of neutralizing antibodies [12]. Thus, both OLGs and molecular systems may be translated too, contributing to vaccine complications and potential adverse effects.

Messenger RNA vaccines, known modifications

To elicit the generation of neutralizing antibodies, exogenously administered mRNA must be heavily engineered to avoid hydrolysis by the extracellular RNAases and detection by cytosolic immune sensors [13,14]. Placing the nucleic acid backbone into lipid nanoparticles (LNPs), hides it from RNAases, while codon optimization, replacing uridine with N1-methylpseudouridine (m1Ψ), renders the vaccine undetectable to sensors [15,16]. Other adjustments were made in the untranslated regions (UTRs) and polyadenylated (polyA) tail to protect and stabilize the vaccine [14,16,17]. Another known change, addition of two proline residues, maintain the S antigen in prefusion conformation to augment immune system exposure [18]. Moreover, aside from m1Ψ, codon optimization includes increased the CG content and possibly G-quadruplex structures to enhance translation [6].

Potential unknown changes

Aside from the reported changes, the mRNA encoded S antigen may have been engineered further to increase efficacy and translation.

Sense codon reassignment?

Pfizer/BioNTech has published the mRNA vaccine sequences, allowing scientists and clinicians to compare codons with the wild type S protein. However, the translated peptides remain proprietary therefore, at this time, it is not possible to rule out sense codon reassignment or introduction of unnatural proteins [19]. This is important as genetic code expansion and incorporation of immunogenic noncanonical amino acids, patented in 2018 (WO2019193416A1), were evaluated for utilization in genetic vaccines [20]. Some unnatural amino acids, especially homoarginine, was associated with heart disease and sudden death therefore, these artificial building blocks may in rare occasions directly contribute to VAERS-recorded events [21-22].

Sugar coating or not?

It is unknown at this time whether the S protein glycans were altered to increase the efficacy of the mRNA therapeutics. However, vaccine-elicited neutralizing antibodies exhibit a distinct glycosylation pattern than post-infection antibodies, indicating possible manipulation [23-25]. This is significant as glycosylation plays a major role in cardiovascular and endothelial homeostasis, providing a potential link to VAERS-recorded events [26,27](Figure 1).

Citation: Sfera A, Hazan S, Sfera DO, Kozlakidis Z. The Matryoshka code of COVID-19 mRNA vaccines: overlapping viral sequences?. Arch Clin Trials. 2022;2(4):1-3.

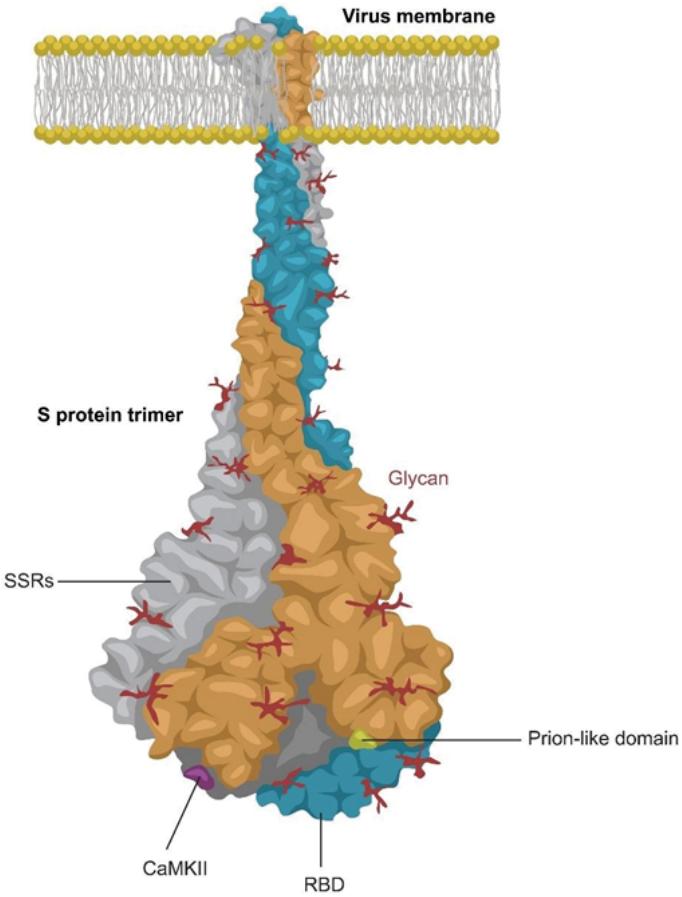

Figure 1. *The overlapping molecular structures and signals in the S protein of SARS-CoV-2 virus. Glycosylation is a viral strategy for successfully exploiting host translational machinery. Vaccination with SARS-CoV-2 S protein lacking glycan shields elicits enhanced responses therefore, glycosylation may have been altered in mRNA vaccines. RBD (receptor binding domain), CaMKII (calcium calmodulin kinase II), SSR(simple sequence repeats).*

The S antigen molecular systems

Several biomolecular systems are present in the S protein of SARS-CoV- 2 that when translated may trigger secondary pathways linked to vaccine adverse effects. These systems include simple sequence repeats, heptad repeats, calcium calmodulin kinase II, and prion-like domains [3-6]. Translation of these molecular structures may lead to new viral variants, pathological cell-cell fusion, and defective proteostasis. These potential links, derived from the viral legacy of overlapping genetic and structural information will be briefly presented below:

Simple sequence repeats (SSRs)

Also called microsatellites, SSRs are present in the genomes of many viruses, including SARS-CoV-2, accounting for a number of the new, emerging variants [28,29]. Trinucleotide and hexanucleotide repeats are the most common SSRs that, aside from their role in viral genomes, contribute to skeletal muscle pathology and neurodegeneration, possibly explaining vaccine-induced neuropsychiatric and neuromuscular symptoms [30,31]. Interestingly, SSRs are influenced by the CG content of nucleic acids which is elevated in COVID-19 mRNA vaccines, linking these therapeutics to the emergence of new variants [32,33].

The DNA mismatch repair factor, MSH3, previously associated with trinucleotide repeats, was also found to function as a sensor for G-quadruplexes therefore, opposing codon optimization [34,35]. This is interesting as a novel study found a proprietary, Moderna-owned, reverse MSH3 sequence that matches the SARS-CoV-2 furin cleavage site, suggesting an OLG [36]. Indeed, to protect the optimized CG content and G-quadruplexes, MSH3 may need to be attenuated or inhibited, explaining the reason this reverse sequence could have been patented (US-9587003-B2).

Heptad repeats

There are two heptad repeats in the S protein of SARS-CoV-2 that assemble into a six-helix bundle to execute membrane fusion [37]. Translation of these structures likely accounts for vaccine-induced pathological cell-cell fusion, that could result in rare post-vaccination events, such as giant cell myocarditis [38,39].

Calcium calmodulin kinase II

Cell-cell fusion can also be promoted by calcium calmodulin kinase II (CaMKII), a system detected in the S antigen of the SARS-CoV-2 virus [4]. CaMKII may promote post-vaccination pathological syncytia, probably accounting for VAERS-reported multinucleated giant cells thyroiditis or myocarditis [4,40].

Prion-like domains

The receptor-binding domain (RBD) of the SARS-CoV-2 virus contains a prion motif that could be translated, leading to pathology (5). Indeed, post-vaccination Creutzfeldt-Jakob disease (CJD) was reported by two separate studies, indicating that the prion motif may get translated [41,42].

In summary

OLG and overlapping molecular structures are common occurrence in viruses and contribute to a number of biological processes. However, such overlapping information may also be translated with the vaccine mRNA, thus inadvertently increasing the odds of pathology. An mRNA vaccine expressing only the RBD may lower the susceptibility for adverse effects.

Disclaimer

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