Potential Mechanisms for Human Genome Integration of Genetic Code from SARS-CoV-2 mRNA Vaccination

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Abstract:

Background

The findings of a sequence embedded in Human DNA that was almost identical to a sequence in the SARS-CoV-2 genome, and the identification of plausible integration of SARS-CoV-2 RNA into human DNA by endogenous reverse transcriptase activity expressed by Long Interspersed Nuclear Element (LINE)-1 (17% of Human DNA) have raised concerns about the long-term safety of messenger-RNA (mRNA) based vaccination. Recent data demonstrate that SARS-CoV-2 RNA sequences can be transcribed into DNA and may be actively integrated into the genome of affected human cells, mediated by retrotransposons. Complementarily, in some SARS-CoV-2 infected patient specimens, there is evidence for a large fraction SARS-CoV-2 sequence integration and subsequent generation of SARS-CoV-2 – human chimeric transcripts.

Results

In this review, the potential role of mobile genetic elements in the etiopathogenesis of cardiovascular, neurological, immunological, and oncological disease and the possibilities of human DNA interference by SARS-CoV-2 vaccination are repositioned. Vulnerable human stem cells as well as gametocytes can presumably be the first targets for unwanted RNA interference. Given the many genetic manipulations of the RNA coding for the SARS-CoV-2 spike glycoprotein in the vaccines, manipulations designed to increase stability and efficiency of spike protein translation, much remains uncertain about the potential disruptions to cellular physiology and homeostasis that could ensue. The predicted consequences pose serious risks to human health that are in need of clarification.

Conclusion

Further toxicity evaluations are urgently needed to quantify potential emergence of interference with canonical DNA processes that could detrimentally impact the mRNA-vaccinated population.

Keywords: SARS-CoV-2 spike protein; reverse transcription; mRNA vaccines; LINE-1; cancer; human DNA integration; human stem cells; etiopathogenesis of disease, p53; polymerase theta.

1. Introduction

A major argument in favor of long-term safety of COVID-19 vaccination, as analyzed by Pardi et al., 2018 [1], was handled by the authors as follows: ‘‘In vaccinated people, the theoretical risks of infection or integration of the vector into host cell DNA are not a concern for mRNA. For the above reasons, mRNA vaccines have been considered a relatively safe vaccine format.’’ However, this seems not to be the case, and safety cannot

be presumed. Embedded within the human DNA is a 94.6% identical sequence (117 bp) of SARS-CoV-2 which is located in chromosome 1p within the intronic region of the netrin G1 (NTNG1) gene, as was demonstrated by Lehrer and Rheinstein [2]. This sequence was discovered during the early phase of the COVID-19 pandemic, and it corresponds to an almost identical orf1β sequence of the SARS-CoV-2 gene, which is close to the spike glycoprotein sequence (the main source of known COVID-19 pathogenesis) [2-4]. Specifically, the human-homologous SARS-CoV-2 sequence matches an orf1β sequence of nonstructural protein (nsp) 14 (which is an exonuclease) and of nsp 15 (which is an endoribonuclease) of the virus [4].

Although SARS-CoV-2 is a single-stranded RNA virus and not a retrovirus, its genomic integration into human DNA is notably feasible in various ways, either a) via endogenous Long Interspersed Nuclear Elements-1 (LINE-1) reverse transcription (RT) [5,6] or b) via the newly recognized human reverse transcriptase, polymerase theta, whose reverse transcription activity is comparable to that of the human immunodeficiency retrovirus (HIV) [7], or c) through defective DNA double-strand break repair mechanisms [8,9]. The resulting DNA copies of the virus are able to become integrated throughout multiple sites of human chromosomes as is described in the fine reviews of Katsourakis and Glifford 2010 [10], and Geuking et al. 2009 [11], although the SARS-CoV-2 RNA is not meant to be reverse transcribed in human cells.

The SARS-CoV-2 sequence copies most frequently integrated into human DNA are those close to 5′ and 3′ untranslated regions (UTRs), showing a preference for sequences neighboring promoters and poly(A) tails [4]. The SARS-CoV-2 LINE-1 RT sequence integration into human DNA seems not to be random but instead is targeting human exon-associated sites [5]. The integration corresponds to the full-size sub-genomic nucleocapsid

(not spike) sequences of SARS-CoV-2, which can be expressed in chimeric (virus-host) transcripts in human cells [5].

The nucleocapsid and spike protein sequences are the richest genomic domains of SARS-CoV-2 in terms of non-synonymous substitutions and thus are of great epidemiologic and medical interest [12]. Notably in this respect, a robust T-cell reactivity (CD4+ and CD8+ lymphocytes) against the nucleocapsid and the spike protein has been recorded in up to 60% of samples from a population unexposed to SARS-CoV-2 during the early onset of the COVID-19 pandemic [13,14]. This important naturally-derived memory immunity against SARS-CoV-2 has been attributed to either previous common cold coronavirus infections [13,14] or previous cross-species transmission of coronaviruses from animal reservoirs to humans [15,16].

The integration of the whole or segmented genomic sequences of yet other retroviruses or RNA or DNA viruses into the human genome is undoubtedly proven, and these can get fixed into chromosomes after several generations [10]. As such, the presence of synthetic mRNAs [1] in the mRNA vaccines, carrying sequences from the pathogenic spike protein of SARS-CoV-2 in close proximity to a poly(A) tail, also means that these have all the prerequisites to become inserted into human DNA. Furthermore, special attention in the manufacturing process of synthetic mRNAs has been paid towards unnatural modifications, such as the conversion of all uridines to methylpseudouridines, aimed to protect the mRNA from degradation [1]. This enhanced longevity within the cell increases the likelihood of reverse transcription and incorporation into DNA via various mechanisms involving mobile elements [17]. Possible mechanisms of human genetic interference and consequences to human health are therefore revisited.

Remarkably, it has been demonstrated in experiments with mice that mammalian sperm are fully capable of translating exogenous messenger RNA into DNA, bundling the DNA up into plasmids, and releasing those plasmids into the local environment during fertilization. A fertilized egg can take up plasmids and retain them throughout fetal development, following birth, and throughout the lifespan. They can even be passed on to future generations. These plasmids can remain autonomous and are able to clone their DNA independently of the human genome [18]. It is therefore conceivable that such a process could take place following mRNA vaccination, which would result in an infant whose cells would have the capability of synthesizing spike protein and whose immune system would view the spike protein as a self-protein, with unknown consequences.

2. LINE-1-Mediated Reverse Transcription of Vaccine mRNA

Researchers in Sweden have conducted an in vitro study on a human hepatic carcinoma cell line (Huh7 cells) exposed to the Pfizer BioNtech BNT162b2 vaccine, specifically examining the question of whether these cells have the capability of converting the mRNA in the vaccine into DNA [19]. The authors found that the cells readily and spontaneously took up the mRNA nanoparticles and responded to exposure by upregulating LINE-1. An immunohistochemistry assay revealed that LINE-1 levels were increased in the nucleus in response to the mRNA nanoparticles. Alarmingly, they verified that a 444 base pair reporter region (amplicon) of mRNA was readily reverse transcribed intracellularly into DNA as soon as 6 hours following exposure.

Tracer studies have shown that the mRNAs in the vaccines enter the lymph system and are eventually taken up by cells in multiple organs, with the liver being second only to the spleen in the concentration detected [20]. It was suggested by Aldén et al. that the liver cells could be exposing spike protein on their surface and in this way inducing an

autoimmune attack on the cells by antibodies [19]. This might explain several observed cases of autoimmune hepatitis in response to the vaccine [21-23].

The mRNA in the vaccines has been engineered to have a long poly(A) tail, which helps both to facilitate translation into protein and increase survival time of the mRNA. However, the presence of a large number of mRNA molecules with long poly(A) tails likely increases expression of poly(A) binding protein (PABP), to serve the needs of these mRNA molecules. PABP has been found to be essential for efficient LINE-1 retrotransposition, and knockdown of PABP greatly decreases LINE-1 activity [24].

LINE-1 proliferation involves a complex life cycle beginning with RNA polymerase II (Pol II) transcription of its mRNA. The mRNA is translated into its two ORFs in the cytoplasm. The ORFs form a ribonucleoprotein (RNP) particle which then transfers to the nucleus for translation of the RNA into DNA and integration into the genome. It is hypothesized that PABP acts as an escort protein that can shuttle the RNP to the nucleus [25]. Thus, the mechanism by which the mRNA in the vaccines increases LINE-1 activity could be through upregulation of PABP.

3. Does Cancer Increase Risk of Retrotranscription of Spike mRNA?

The epigenetic modification involving methylation of cytosine in CpG islands is an important factor in regulating gene expression. It is estimated that more than 90% of all 5-methylcytosines lie within the CpG islands of the transposons, i.e., the long and short interspersed nucleotide elements (LINEs and SINEs). In fact, the extent of LINE-1 methylation is regarded as a surrogate marker of global DNA methylation. Hypomethylation of the promoter of LINE-1 activates its expression. High levels of LINE-1 activity are associated with many tumor tissues, including breast cancer, esophageal cancer, colon cancer and lung squamous cell carcinoma. LINE-1 can mediate

the inactivation of tumor suppressor genes, and it promotes cell proliferation and invasion [26].

The experiment by Aldén et al. demonstrating reverse transcription of spike mRNA involved human hepatic carcinoma cells grown in culture. Liver cancer accounts for 9% of all cancer worldwide, and 80% of the cases are diagnosed as hepatocellular carcinoma. Intriguingly, a link has been found between LINE-1 retrotransposons and hepatitis B or hepatitis C infection. Several LINE-1 chimeric transcripts with host or viral genes are found in hepatitis virus-related hepatic carcinoma. Furthermore, endogenous LINE-1 retrotransposition was demonstrated to activate oncogenic pathways [27]. These observations suggest that the mRNA vaccines could induce or accelerate the advancement of hepatocellular carcinoma (HCC) in exposed humans through a similar process, by upregulating LINE-1 activity. In this respect, development of HCC is linked to Hepatitis C virus (HCV -- a positive stranded RNA virus) chronic infection [27]. Through LINE-1 alternative retrotransposition mechanisms [28], it seems feasible for HCV RNA to become reverse transcribed. Complementarily, the production of cDNA clones from HCV RNA has been achieved in primate animal models [29], and sequences of the HCV genome are readily detected in other mammalian species [28].

Furthermore, when the degree of hypomethylation of LINE-1 DNA in hepatic tumor cells was compared with the adjacent normal cells, the results (48.6% vs 71.7% methylated) were highly significant ($p < 0.0001$) [30]. A study on colon cancer showed that LINE-1 was hypomethylated even in normal tissue cells adjacent to the tumor in association with worse outcomes among cancer patients [31]. Hypomethylated and highly expressed LINE-1 has also been found in autoimmune diseases such as systemic lupus erythematosus, Sjögren's syndrome and psoriasis [26]. Since exposure to the

SARS-CoV-2 RNA caused an increased expression of LINE-1 in infected patients [5], this also suggests that the genetic vaccine mRNAs may cause an increased risk of developing cancer or autoimmune disease via possible LINE-1-mediated DNA integration. This can also be expected to accelerate progression of these aforementioned diseases.

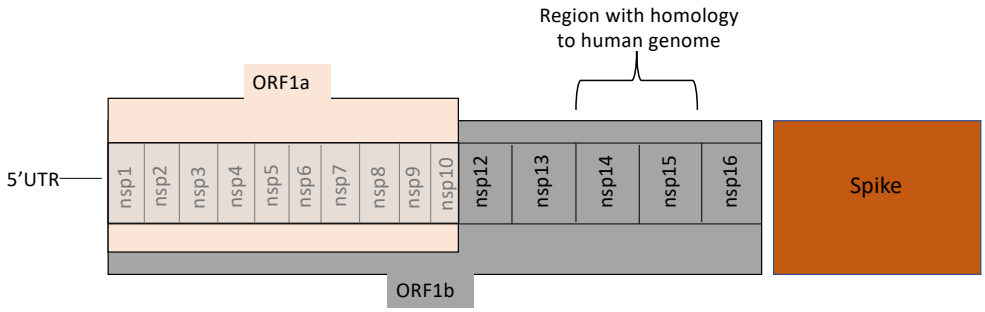

Figure 1: Illustration of the segment of the SARS-CoV-2 genome that is nearly identical to a human gene sequence. Adapted from Figure 2, Rastogi et al., 2020 [32].

The Mobile Genetic Elements and Neurological Disease Etiopathogenesis

The segment of SARS-CoV-2 that is nearly identical to a human gene sequence is within nsp14 and nsp15 in the viral genome, with only nsp16 (a 904 bp sequence) separating it from the spike protein sequence, as schematized in Figure 1. [1,4]. This segment is embedded within the orf1β of SARS-CoV-2. Other viral ORFs, encoded as endogenous

elements, are expressed as mRNAs in human cells [10]. The human genomic sequence that is homologous to the SARS-CoV-2 genome is located within the *NTNG1* gene [2, 4]. Importantly, disturbances of neuronal development associated with genetic anomalies within the *NTNG1* and *NTNG2* genes are proving responsible for the pathophysiology of schizophrenia [33,34].

A plethora of RNA-binding proteins are critically involved in transcription control [35]. Even though only a small fraction of the synthetic RNA gets into cells, the presence of synthetic mRNA in vaccines even at concentrations as low as 30 ug and 100ug (a minimum of 40 trillion synthetic mRNA molecules) may produce RNA-protein-binding complexes that control transcription. For example, this is important when the binding protein can be the topoisomerase *3β* (with biological properties to control mental, aging and neurodevelopment functions), as this specific enzyme forms a highly conserved and medically important complex with yet another protein, Tudor-domain containing 3 (TDRD3) [36]. This powerful complex may interact with histones, single-stranded RNA, DNA, translation factors, and polymerase II. This may cause non-physiological neurodevelopment and aging defects in humans [36].

During studies to discover SARS-CoV-2 and human protein-protein relationships, 332 interactions of high confidence were revealed between the two species [1]. These interactions actually demonstrated the promising efficacy of chloroquine and an antipsychotic drug haloperidol against SARS-CoV-2. Nevertheless, these numerous protein-protein interactions complicate even more the possible protein expression of SARS-CoV-2 sequences in human DNA and their interactions through Human Endogenous Retroviral, Alu and LINE-1 genomic DNA-encoded reverse transcriptases,

and other human endogenous proteins [37]. Such interactions have been shown to have severe consequences in neurological diseases [38].

This may be even more important for patients already infected with SARS-CoV-2 who then receive the spike protein sequences in mRNA vaccines and have already reverse-transcribed SARS-CoV-2 sequences scattered throughout sensitive organs such as the central nervous system [1,4,5]. This may be highly consequential for those patients who also suffer from pre-existing neurodegenerative diseases. [38]. Already, there are emerging reports regarding COVID-19 mRNA vaccination association with acceleration of Parkinson's disease [39,40] and prion disease [41].

Recent investigations reveal the persistent presence in the blood up to 15 months post infection of SARS-CoV-2 spike S1 subunit (S1) that is able to cross the blood brain barrier, likely within exosomes, in patients suffering from post-acute sequelae of SARS-CoV-2 infection [42]. However, this finding requires further investigations as to whether the S1 protein itself is persistently carried by non-classical CD14lo, CD16+ monocytes for a long period of time or if instead the S1 presence is the result of endogenous DNA production, as the possibility of whole virus persistence in cells has been excluded in this study [42]. Retrotransposition may also explain the enduring presence of both mRNA and spike protein in lymph node germinal centers up to 60 days post-vaccination [43].

4. mRNA SARS-CoV-2 Vaccination can Cause Interference with Human Genomic DNA like Other Viral RNAs

RNA molecules have the ability to spontaneously modify their sequences and, even when fragmented, to direct the synthesis of their respective copies [44]. RNA recombination [45] and transmissibility via sperm [18] or via metathesis reactions to the next generation of cells is one of the major obstacles to overcome in mRNA technology application for

infectious disease vaccination [1]. Long Terminal Repeats (LTRs) within Human Endogenous Retroviruses (HERVs) [37,46] contain the necessary sequences of promoters, enhancers, and poly(A) tail signals to reverse transcribe a foreign RNA sequence to a dsDNA and thereafter, as for the SARS-CoV-2 RNA, to integrate multiple fragments into various human chromosomes [1,4,7].

The LTRs therefore have all the necessary machinery, apart from necessary enzymes for reverse transcription [7] and integration into human DNA, to code for viral envelope, nucleocapsid and matrix capsid [38] and potentially produce new recombinant viral particles having chimeric (host and viral) sequences. Similar chimeric sequences were detected in cell lines infected with SARS-CoV-2 [5]. Since LINE-1 retrotransposons are amplified during early embryonic life [47], this constitutes a likelihood for circulating dendritic cells derived from hematopoietic stem cells and reproductive cells (oocytes and sperm cells) to be affected. This is true even with small dosages of mRNAs in vaccines, where long-lived SARS-CoV-2 spike protein RNA sequences could be reverse transcribed upon entering the cell, and subsequently be encoded into germ-line libraries. This can cause additional production of spike protein sequences beyond those initially intended by endogenous expression [1]. In this respect the assembly of virions carrying chimeric SARS-CoV-2 sequences is a probable long-term consequence [5,7]. Functional insertions within the HERV sequences, other than evolutionarily driven [10], can awaken the otherwise epigenetically silenced HERV and LTR genes. Of foremost concern is that these can become active and play a causative role in autoimmunity, tumorigenesis and other disease progressions [48].

By this kind of DNA interference, which is highly plausible with SARS-CoV-2 mRNA sequences [1,4,5], regions of DNA like those of HERV-K elements, called HERV-K human

mouse mammary tumor virus like-2 (HML-2) regions, can also be awakened [46,48]. Such a phenomenon has already been proven for other non-retroviral RNA viruses [10] as well as other coronavirus sequences [4]. The HERV-K (HML-2) region alone contains more than 90 provirus segments scattered throughout the whole of transcriptionally active human DNA, and these can be carcinogenic, triggering melanomas and teratocarcinomas [49]. These unfortunate genetic events can happen simply by disturbing natural anti-cancer host defense mechanisms, developed over millions of years from co-evolution of host and viral genetic material exchange and dissemination throughout the human genome as a line of health defense [49].

5. **Potential for Inducing Oncogenesis and Metastasis: The Role of Stem Cells**

To obtain optimum results of protein expression during development of intradermal vaccination with synthetic mRNAs, at a minimum a 900 bp macromolecule sequence was needed [50]. The average molecular weight of ribonucleotide monophosphates is 339.5 g/mol (MW) [51]. This makes the doses of 30 μg and 100 μg of synthetic mRNA vaccines at first glance seem extremely low to be capable of genetic interference within human cells [50]. For non-dividing cells, it seems that the risk of insertional mutagenesis is low [50]. However, the synthetic mRNAs, even within minutes post-vaccination, rapidly disseminate from the injection site to the neighboring draining network of lymph nodes [52]. The widespread niches of lymph nodes throughout the whole organism contain quiescent undifferentiated precursor stem cells receiving proliferation signals under stress conditions, and hence mitotic division of these cells is elevated [53].

Healthy human stem cells (HSCs) have an overwhelming capacity for accelerated mitotic division that confers their enhanced ability to transform into cancer stem cells in comparison to stem cells from other species. In fact, it was due to their unique capabilities

to regenerate and form resemblances of *ex vivo* tissues that the whole of RNA editing technology was built to serve curative purposes [54]. Notably, the RNA editing of HSCs pursued in the laboratory is passed robustly and with high frequency from parental HSCs into the next generations of cells that then become cancerously modified stem cells. Laboratory studies strongly suggest that the synthetic mRNA molecules contained in the current mRNA vaccines could induce pathological changes in the established cellular mechanisms for pre-mRNA modifications taking place within the nucleus of HSCs within the lymph system [53]. RNA editing (epigenetic modifications and post-transcriptional regulation) is a highly sensitive process, errors within which can establish malignancy in stem cells. Stem cells have a highly vulnerable orchestration of genetic events in response to both intrinsic (within the cell) and extrinsic (out of the cell) factors [54]. Also, the emergence of malignancy from previously healthy stem cells has been proven to be easily induced by endogenous microRNA (miRNA) interference (epitranscriptomic regulation) during mRNA editing [54]. Additionally, as the stimulation of activation, differentiation and proliferation is a common task for immune cells and other stromal cells located in lymph nodes [53], the risk of DNA interference or epigenetic disturbance by even one synthetic mRNA macromolecule entering the cell cannot be excluded.

6. Spike Protein Induces Oncogenic Signaling via JAK/STAT3 Pathway

A case can easily be made that a stem cell in a lymph node is vulnerable to oncogenesis through the influence of the spike glycoprotein, which is being obligatorily produced from the mRNA in the vaccine. Many studies have shown that the spike protein alone is capable of inducing overexpression of the pro-inflammatory cytokine interleukin-6 (Il-6) [55-57]. This cytokine in turn induces tyrosine phosphorylation of STAT3, which then migrates to the nucleus to induce an inflammatory response [55].

In the nucleus, STAT3 binds to and activates promoters of a broad panel of genes encoding proteins that induce cellular proliferation, a key step towards tumorigenesis [58]. Hyperactivation of STAT3 occurs in many types of cancer, including acute myeloid leukaemia, multiple myeloma, and solid tumors of the bladder, bone, breast, brain, cervix, colon, oesophagus, head-and-neck, kidney, liver, lung, ovary, pancreas, prostate, stomach, and uterus. [58].

The JAK/STAT3 pathway has been shown to promote the conversion of human pre-leukemia stem cells into acute myeloid leukemia stem cells. The mechanism involves activation of enzymes that deaminate adenosine in double-stranded RNA, converting it to inosine. This class of enzymes is called the adenosine deaminase acting on RNA (ADAR) enzymes, and they are strongly linked to cancer [59]. They induce an A-to-I transformation in double-stranded RNA that ultimately results in a missense encoding of adenosine as guanidine. Priming of Il-6 through a recent mRNA vaccine could accelerate the mutation rate in the spike protein mRNA during a subsequent active infection with SARS-CoV-2, directly through upregulation of ADAR enzymes. This could explain the rapid emergence of resistant variants of SARS-CoV-2 in recent months, following an aggressive vaccination initiative at the population level [60].

A-to-I editing of double-stranded RNA is a post-transcriptional regulatory mechanism that plays an important role in cancer. A major place where A-to-I editing takes place is the 3′-UTR region of mRNA molecules. Importantly, such editing leads to a shortening of the 3′-UTR segment, which often results in the removal of binding sites for miRNAs that suppress protein synthesis. In this respect, such editing increases protein expression of the affected gene, often leading to carcinogenesis [61].

The HSCs have the potential to differentiate and become literally any kind of cell in the mature organism, and the not-sufficiently-guided genetic interference of stem cells may lead to diseases such as hematopoiesis disorders as well as cancer [62]. Lymph-circulating tumor cells are commonly present in patients with diagnosed malignancies. The presence of these cells does not, of course, depend upon an established diagnosis and should be expected to be found in patients with an undiagnosed malignancy as well. The presence of these cells confers a clinically important metastatic potential as compared to the blood-circulating malignant cells that have escaped from primary tumors, and this process can be augmented by any RNA interference. As the synthetic mRNAs tend to disperse and accumulate in regional lymph nodes [52] and the lymph-circulating malignant cells have a stem cell cycle mosaic of proliferation [48,54] and use the entire lymphatic system to travel, then the risk of augmented metastatic potential can also be considered as elevated in these cases [62].

7. Spike Protein, Inflammation, Syncytia, DNA Damage and Senescence

In a cleverly designed laboratory experiment, Meyer et al. explored the notion that exposure of the pulmonary epithelium to the spike protein can lead to the release of mediators that drive endothelial dysfunction [63]. These researchers demonstrated that spike-transfected human A549 epithelial cells released inflammatory molecules that are characteristic of a senescence-associated secretory phenotype (SASP), along with a 3-fold increase in reactive oxygen species (ROS). Furthermore, ROS levels were increased approximately 2-fold in endothelial cells exposed to the culture medium taken from the spike-producing A549 cells compared to empty plasmid-transfected control cells. Hence, endothelial cells respond to signaling from spike plasmid-transfected epithelial cells through a “bystander senescence response” that can lead to endothelial damage via a

paracrine process. Cellular senescence was also associated with an increased level of endothelial adhesion molecules promoting leukocyte tethering to the vascular wall. Such tethering is a first step towards leukocyte extravasation and subsequent tissue invasion and inflammation.

These results are consistent with those of another study that investigated the response of cultured bronchial epithelial and endothelial cells to spike-protein transfection, which also demonstrated that the cells responded with increased ROS levels triggering an inflammatory response and ultimate apoptosis [64].

A remarkable series of papers by a large team of researchers in China have demonstrated indisputably that the spike protein causes cells that have ACE2 receptors (such as the pulmonary epithelial cells) to form multinucleated giant cells, known as syncytia, via cell-cell fusion [65-67]. This cell fusion response depended upon protease-dependent cleavage of the spike protein into S1 and S2, and further cleavage of the S2 subunit at the S2' site [66] [4]. These syncytia eventually succumb to cell death by pyroptosis, enhancing the inflammatory response.

Furthermore, multiple micronuclei were detected within the syncytia, and these micronuclei were associated with γH2Ax (H2Ax with phosphorylated Ser139), a highly precise and very early marker for DNA damage [67]. These authors wrote: "Together, these results suggest that the syncytial micronuclei are the sites succumbing to genomic instability and DNA damage." [67] The increase in γH2AX detection due to spike protein expression in A549 epithelial cells is accompanied by an increase in $p16^{INK4A}$ tumor suppressor and p21 oncogenic proteins [63]. The fact that the spike protein also induces upregulation of LINE-1 should raise concern for the potential for reverse transcription of

spike protein mRNA in the context of the formation of syncytia invoking DNA damage repair mechanisms.

The so-called "cGAS-STING DNA sensing pathway" is an important biological pathway that responds to cytoplasmic chromatin fragments (CCF) and activates a type-I interferon response. Cyclic GMP-AMP synthase (cGAS) is the DNA sensor that then causes the endoplasmic reticulum protein, stimulator of interferon genes (STING), to trigger the interferon response. It was demonstrated that the cGAS-STING pathway was a key player in the induction of the type I interferon response in cells transfected with spike protein [66] [4]. The authors of a perspective article on the cGAS-STING pathway wrote: "While short-term inflammation triggered by the CCF-cGAS-STING pathway is required for immune clearance of senescent cells, chronic inflammation mediated by SASP is destructive, resulting in tissue damage and even tumorigenesis." [68] A review paper with the provocative title, "DNA Damage – How and Why We Age?" argued that excessive activation of the DNA repair mechanism due to persistent DNA damage may be the primary cause of accelerated aging and the associated diseases [69].

8. DNA Repair Mechanisms: An Active Role for Polymerase Theta

Normally, when a cell is infected with a virus, it immediately launches type I interferon signaling upon detection of viral RNA. One of the important consequences of the subsequent signaling cascade is the upregulation of the tumor-suppressor gene p53. P53 induces cell cycle arrest upon detection of double-strand DNA breaks, thus protecting the cell from severe genetic defects during replication and thus is tumor-supressing. Multiple DNA repair strategies are available to repair the breaks so that replication can resume. P53 also arrests viral replication, thus slowing production of multiple copies of the virus to further the spread of infection [70]. Any DNA double-strand break (DSB)

opens up the opportunity for a chromosomal translocation, where the two fragmented pieces re-attach to different chromosomes. This can result in both missing genes and extra genes, which can profoundly disrupt chromosomal integrity, causing a progression towards cancer. Thus, it is imperative to repair the break before these potentially catastrophic genetic alterations can take place.

Gene editing is a technology that gives scientists the ability to change an organism's DNA, by altering, removing or inserting genetic sequences at a specific location in a genome. The most well-known gene editing technology is CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats - CRISPR-associated protein 9). This technology is borrowed from a bacterial capability to combat bacteriophages. CRISPR sequences, originating from bacteriophages, are found in half of sequenced bacterial genomes and in nearly 90% of genomes from archaea [71]. Cas9 uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the strand in the CRISPR sequence.

While CRISPR/Cas9 is considered to offer precision control over the location in the DNA sequence that is modified, this has turned out to not always be true. The technology begins by introducing a DNA double-strand break, and it relies on standard cellular methods to repair the break. Eukaryotic cells have acquired multiple mechanisms to repair DNA breaks, depending on the stage of the cell cycle. So-called homologous recombination (HR) is very accurate, but it depends on the availability of a DNA template as a guide, which is only available during the later G2 and M stages. Non-homologous end-joining (NHEJ) comes into play during earlier stages of the cell cycle (G0, G1 and S). Its repair process is more prone to introducing transcription errors. A third repair mechanism, termed microhomology-mediated end joining (MMEJ), has only

been recognized as an alternative mode of repair in the past few years [72]. It involves first annealing two microhomologous regions of the two broken strands, and then filling in the gaps through DNA synthesis using a DNA polymerase. A significant part of the original sequence gets removed through this process, and thus it is an error-prone solution.

Polymerase theta (PolΘ) is an important DNA repair enzyme involving double-strand DNA breaks using MMEJ, also known as "synthesis-dependent end joining" and "theta-mediated end joining." As outlined above, the method causes the insertion of additional sequences at joining sites as well as deletions - so-called "indels." It is not expressed in most tissues, with tumors being the notable exception. It is upregulated in association with many cancers, including stomach, lung, and colon cancers, breast cancer and ovarian cancer, and its overexpression is a prognosis for poor clinical outcomes [73]. PolΘ is a key driver of genome evolution and of CRISPR/Cas9-mediated mutagenesis [74].

We hypothesize that the mRNA vaccines coding for spike protein set up a situation in a transfected cell, particularly one that is in a proliferative state, that could be highly susceptible to severe chromosomal aberrations. Because the technology involves extensive modifications to the original viral mRNA to conceal its viral source, it achieves a "stealth" entry into the cell without provoking a normal type I interferon response [75]. The cell immediately launches efficient translation of the mRNA to produce abundant amounts of spike protein. The spike protein causes severe DNA damage, including double-strand breaks, as described previously. This genetic stress does launch a type I interferon response, but it is delayed such that significant damage takes place before p53 is sufficiently upregulated. Furthermore, DNA-damage induced interferon β is directly

implicated in cell senescence and inhibition of stem cell function associated with accelerated aging [76].

A paper aptly titled, "Repair of G1 induced DNA double-strand breaks in S-G2/M by alternative NHEJ" showed, using CRISPR technology to disable p53, that DNA breaks introduced during G1 could later be repaired by polΘ, after the cell cycle had advanced to S-G2/M phase [77]. By disabling the less promiscuous NHEJ repair pathway, they allowed the cell cycle to progress after the break had occurred, leading to the generation of multiple aberrant chromosomal rearrangements while promoting overall cell survival. Importantly, G1-induced broken DNA ends generate chromosomal translocations at a high frequency during the S-G2/M phases, indicating that the broken DNA ends have lost the ability to reconnect during cell cycle progression. Notably, PolΘ is unable to repair DNA breaks during G1 phase [77]. As stated by W. Feng et al., "Pol θ/TMEJ addiction is associated with increased levels of replication-associated DSBs, regardless of the initial source of damage." [78] This implies that excessive DNA damage induces upregulation of polΘ. This suggests that cancer cells and proliferating immune cells transfected with the spike protein would suffer from an accelerated rate of genetic mutations, leading to cancer progression.

A study published in 2021 revealed the unexpected discovery that PolΘ is capable of reverse transcribing RNA into DNA [7]. In fact, PolΘ exhibits a significantly higher velocity and fidelity of deoxyribonucleotide incorporation on RNA versus DNA. It can undergo a remarkable structural transformation in order to maintain productive interactions on DNA/RNA templates. It can accommodate a full RNA-DNA hybrid within its active site, and efficiently transcribe template ribonucleotides into DNA, thus promoting RNA-based DNA repair. PolΘ appears to be unique among human

polymerases in its ability to reverse transcribe RNA, with an efficiency equivalent to that of the retroviruses. It is therefore possible that PolΘ can reverse transcribe vaccine-transfected mRNA into DNA and integrate it into the genome at DNA break sites.

All of these considerations are summed up in the flow chart shown in Figure 2.

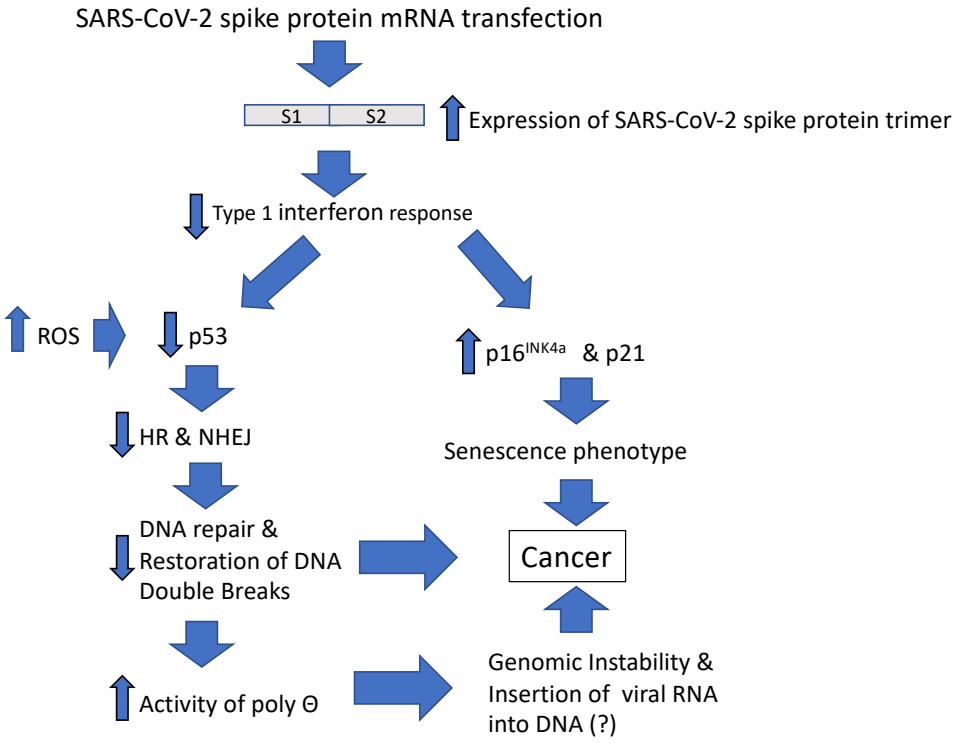

Figure 2: Schematic of sequence of events hypothesized to play out in response to cellular uptake of the mRNA sequences in the SARS-CoV-2 mRNA vaccines, particularly for cells with an active cell cycle. See text for details.

9. DNA Break Repair Mechanisms: When RNA meets DNA.

It was postulated long ago that, apart from retroviruses that have the capability to become inserted into human DNA by reverse transcription, the genetic material of all other RNA

viruses cannot become inserted into DNA under any circumstance [1]. However, experimentally, this has long been proven not to be the case. In 2009, Geuking et al. showed that an otherwise unwarranted genetic recombination could occur between the lymphocytic choriomeningitis RNA virus and the endogenous intra-cisternal A-type (IAP) retrotransposon, and that this led to reverse transcription of the exogenous viral RNA [11]. This exogenous RNA was finally inserted by means of its complementary DNA into the recipient DNA, together with the IAP element. Since this important finding, as the authors declared, it became warranted to properly investigate any potential interaction with retroviral elements before RNA viruses could be used therapeutically to insert new genetic material. Retroelements are active remnants of the RNA-to-DNA world transition that occurred millions of years ago on earth. The active interaction of all RNA viral genetic elements with eukaryotic DNA is now a readily occurring phenomenon sustaining human biodiversity [17].

Compounding the problematic potential of viral or vaccine mRNA integration into host cell DNA is the potential destructive impact of the spike protein itself on DNA. Double-stranded DNA breaks are a severe type of DNA damage, and they carry the greatest risk of initiating a malignant transformation in affected cells' progeny. BRCA1/2 and p53 orchestrate highly complex DNA repair processes specifically directed toward repair of dsDNA breaks [79].

The potential for double-strand DNA breaks brought about by the spike protein seems compelling, given the evidence of micronuclei and syncytia formation in exposed fibroblasts [65-67]. G1 and G2/M checkpoint malfunctioning is coupled with the subnuclear inhibition of the formation of BRCA1 and 53BP1 foci [9,80]. An in vitro study has shown that the S2 subunit of the spike protein interacts with both BRCA and p53,

suggesting that it could interfere with their anti-cancer function [81]. The cells affected by SARS-CoV-2 spike protein continue with their mitotic division with unresolved DNA breaks in chromosomes [82]. This creates a serious case of recombinogenic events as the cells continuously undergo transcription and replication, where the formation of co-transcriptional R loops is imminent if not regular [8].

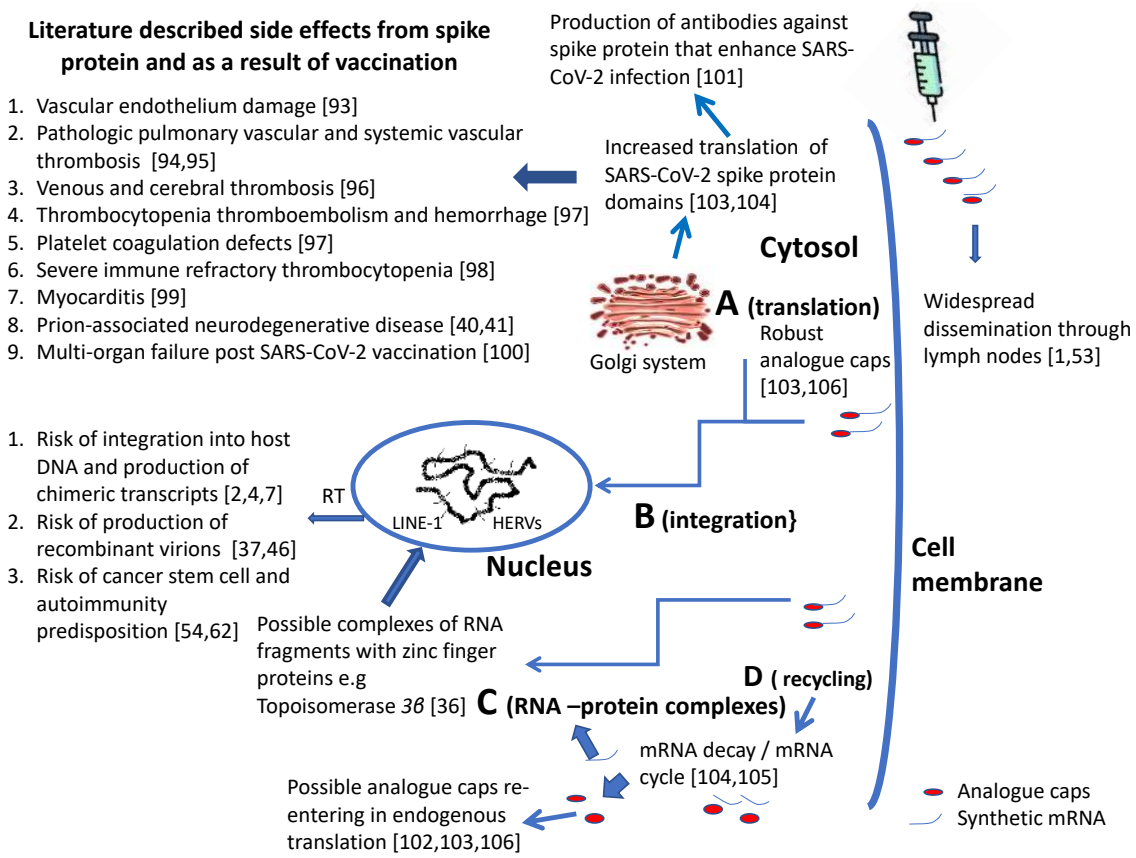

Figure 3. Multiple ways that mRNA in SARS-CoV-2 vaccines may induce pathology and genetic side effects in dividing human cells and the organism. (A) Spike protein translation. The enhanced translating spike protein mRNAs result in serious side effects, verified in publications, (B) Genomic integration; The LINE-1, polymerase theta and HERV reverse transcriptase autonomous retrotransposons can possibly reverse transcribe within more vulnerable dividing cells (stem cells in lymph nodes) and produce chimeric sequences of host and virus spike protein fragments and new virions.

Genetic disturbance of otherwise silent HERVs may produce neurodegenerative disorders and cancer. (C) Recycling; Continuous mRNA decay and recapping may re-allocate robust analogue caps to endogenous mRNAs. (D). RNA-Protein complexes; Fragments of spike protein mRNAs may form protein complexes with endogenous nucleases to produce DNA interference. LINE-1: Long Interspersed Nuclear Elements 1; HERVS: Human Endogenous Retroviruses; RT: Reverse Transcription.

Recently published literature on SARS-CoV-2 spike protein driven cellular and tissue injury reveals a large number of COVID-19 vaccine injury syndromes. [40-41,93-100,102-106]. Many of these injuries can be expected if the mechanisms described in this paper are taking place. Figure 2 summarizes our findings by describing multiple ways that mRNA in SARS-CoV-2 vaccines may induce pathology in dividing human cells. There is strong evidence that the spike protein itself induces DNA damage and subsequent DNA repair mechanisms. It also causes increased expression of LINE-1, which is capable of converting the mRNA to DNA. Chimeric transcripts can emerge from the processes that ensue in the nucleus. RNA-protein complexes derived from the vaccine mRNA lead to unpredictable sequelae. These processes combined suggest exposure to mRNA coding for the Spike protein is potentially oncogenic, particularly in those who already have polymorphisms in p53 and or BRCA as well as those with latent or manifest malignancy.

10. **Conclusion**

Recent discovery of SARS-CoV-2 genome integration [**1**,**4**,**5**,19] through a mechanism involving LINE-1 or polymerase theta raises great concern regarding possible unwanted durable incorporation of spike protein sequences into the human genome. Human DNA interference by synthetic mRNAs in vaccines is more than simply a possibility, since

reverse transcription of code from COVID-19 vaccine mRNA has been demonstrated in human hepatoma cell lines. Since their encoded sequences are specific for SARS-CoV-2 spike protein, and these can also be integrated into human DNA, the resulting pathogenesis due to molecular vaccination requires an explicit evaluation through genotoxicity research. In addition to the pathogenic potential of endogenously (DNA) encoded spike proteins, we have shown that activation of the cellular enzymatic networks that carry out this DNA integration entail their own distinct and multifaceted pathogenic potential. These risks are expected to be highest in specific vulnerable populations, namely individuals during the developmental phase (children) and patients suffering from malignancy, autoimmune disease, cardiovascular and neurological disease, and genetic disorders. We recognize that it is speculative to suggest that vaccine mRNA could initiate the broad range of pathological events we describe. Given the extensively documented potential for both endogenous (human retroviral) and exogenous (viral) RNA to trigger these events, though, relevant investigations are urgently needed, given the large number of individuals who have been administered one or more of mRNA products coding for the SARS-CoV-2 spike protein.

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of supporting data

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' Contributions

AK, GN and SS all contributed substantially to the writing of the document. AK, PM, GN, and SS all participated in multiple revisions. GN prepared Figure 1. AK and SS prepared figures 2 and 3. All authors have read and approved of the final version of the manuscript.

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In memory of Mrs RODAMANTHI KYRIAKOPOULOY BA BSc.

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