CRISPR: The biotechnological revolution – risks and achievements public health should know

Gene editing has brought public health the infamous mRNA vaccines and promises improvements, particularly in cancer therapy.

Rapid developments in science revolutionized biology and related disciplines. Especially genomics jumped ahead, making use of fascinating technical advancements. The public seldom takes in the achievements. Genetic engineering for improving crop production is an exception and is heavily rejected (1). Unlike the rejection of engineered food products, similar suspicions against mRNA vaccines were missing due to the pharmaceutic industry, which avoided letting the public realize that the entirely new type of vaccine relates to genetic engineering. It was feared that this might ruin the acceptance of the product. It remains to be seen how long the public can keep believing that mRNA vaccines are not much different than those used before.

The anti-vaccination front argues that mRNA injections from Moderna and Pfizer against the Covid-19 virus are no vaccines but, in reality, 'gene therapy' (2). According to the U.S. Food and Drug Administration (FDA) definition, those calling it 'gene therapy' have a point. The definition of the FDA reads, 'Human gene therapy seeks to modify or manipulate the expression of a gene to alter the biological properties of living cells for therapeutic use' (3). The human cell is stimulated to produce the spike protein of the SARS-CoV-2 virus. According to the FDA definition, mRNA vaccines are gene therapy (4).

The controversial mRNA vaccine should be a lesson learned for public health.

The worldwide use of mRNA vaccines is now heavily condemned in the Western mass media because of the much suffering and death that it caused. Even the German Minister of Health admitted in March 2023 that those vaccines had severe side effects. It is criticized that the world was exposed to a new method of vaccines in which impact and risk were not sorely investigated. However, vaccination in several countries is still ongoing. The Swiss government presently announced that it has stopped using the products. It would be the mission of public health to inform and guide the public on this issue worldwide.

This blog repeatedly commented on the debatable mRNA vaccines.

This blog, based on references of the international literature, recurrently made its followers aware of the controversial developments, especially against the widespread use of mRNA vaccines (5-8). However, it is doubtful that most of those working in the field of public health were aware, while bravely accepting a shot of a Moderna or Pfizer vaccine, of what the four letters 'mRNA' stand for. 'Messenger ribonucleic acid (mRNA)' and 'deoxyribonucleic acid (DNA)' are key elements of our genome and existence. The more we know about this system, the more complex it appears to be, and by no means do we already know precisely how it works.

But the essence of what omics hold for health and disease for the population must be of concern. It's not the village health worker or the public health nurses in the field, but public health

academics in the government and the universities should not miss relevant advances in genetics, which is also of relevance for the public (9).

Remembering the Watson-Crick-Spiral.

Remembering basics in genetics at school, one might know that nucleotides are letters of DNA and RNA and that complementary base pairs of DNA are formed through hydrogen bonds. Guanine binds with cytosine, adenine with thymine, and uracil instead of thymine as far as RNA is concerned. The base pairs are attached to a sugar and a phosphate molecule and form nucleotides arranged in two long strands, the 'double helix'. The information of the genetic code stored in the DNA is translated by RNA into protein. Transcription of RNA results in mature RNA, which is channeled out of the nucleus, and translation occurs at the ribosome where, by the action of tRNA, amino acids are finally formed into proteins (10). For more details, refer to the references or enroll in a short e-learning course in English or Thai.

What is 'CRISPR"? – Scientists learned from bacteria.

Two lady scientists, Jennifer A. Doudna, and Emmanuelle Charpentier, received the Nobel Prize for Chemistry in 2020 for their work on CRISPR Cas9 (11). Both scientists' scientific careers are described for Charpentier in Nature and Doudna in Science (12, 13).

'CRISPR' is the newest method for gene editing. CRISPR stands for 'clustered regularly interspaced short palindromic repeat' (14). The strange idiom for CRISPR incorporates the word 'palindromic', which means a figure or an expression such as 1881, or the word such as 'dad'. To read the two examples backwards, the meaning remains the same. CRISPR enables to add of genome sequences into the DNA strand, and the expression hints toward that mechanism and the RNA involved.

What Streptococcus pyogenes taught scientists.

Scientists learned from bacteria such as Streptococcus pyogenes how to manipulate genes, which is considered a 'biotechnical revolution' in genetic engineering (15). Bacteria use CRISPR to develop adaptive immunity by splitting genetic material from invading enemies such as bacteriophages or viruses through endonucleases. The nucleases connected to the invading DNA sequences of the foreign genomes by cutting 'snippets' of the foreign DNA and integrating them into its own genome. Now the bacterium remembers the attacker and defends it the next time the system known as CRISPR-Cas9 will work again. The place is marked where the CRISPR-Cas9 is located at the genome of the bacterium. The design inhibits the system to start cutting the bacterium's own genome.

It happens, and that's the amazing issue: the system not only works in the bacterium but can be used universally in cells, organisms, animals, and plants. Bacteria got several different types of the method, but presently the type II CRISPR-Cas system, the CRISPR-Cas9, is the most often used. Other bacteria have different Cas9 variants (16) (Table 1).

Let's think of scientists who aim to insert into a single DNA strand sequence another DNA sequence intended to be added. As given above, the process needs the Cas ('Cas' stands for associated) protein, acting as an enzyme. The enzyme CRISPR-Cas9 needs a guide sequence to be directed where at the double-stranded DNA the cut should be. The guide is a double RNA structure composed of the 'tracrRNA:crRNA'. (tracer RNA) and the specific CRISPR RNA), together, the component is called single guide RNA or sgRNA. The 5' site (where the split from the Watson-Crick spiral is made) is next to a PAM (proto adjacent motif) sequence. The sequence of PAM for CRISPS-Cads is 5'-NGG-3,' and N can be any nucleotide base. An RNA structure at the 3' site binds to the Cas9 and completes the process (13, 14, 16).

The double-stranded DNA cut process.

The single-stranded DNA cut processing was followed by a double-stranded one. The mechanism, for example, is used for the Knockout mice technology. One major application of the Knockout mice (K.O.) system serves to study the complex genome influence on the metabolism. An example is given in this blog by describing how to study the metabolic regulations and interferences of adiponectin. A genetically engineered mice were applied, overproducing adiponectin but deficient in leptin (ob/ob) (17).

The CRISPR gene knockout process differs from the single-stranded DNA cut in that a double-stranded DNA break (DSB) is achieved through the Cas9-CRISPR-ribonucleoprotein (RNP). The Cas9 nuclease is attached to an engineered single-guide RNA molecule (sgRNA). After cutting out the intended eliminated DNA part of the genome, the 'smart' endogenous repair system, consisting of a DNA-dependent protein kinase, joins the double-stranded DNA ends without the need for sequence homology. In the CRISPR terminology, the process is named 'nonhomologous end joining (NHIJ)'. To add a function, the 'homology-directed repair (HDR)' pathway provides the repair template (18-20) (16) (Figure 2).

The K.O. system and further developments

Creating animal models for researching genome functions is not new. CRISPR KO, however, added valuable benefits. The method is more accurate and reduces the time to have the laboratory animal available from about one year to four weeks. CRISPR-Cas9 even works in zygotes and can be applied through oviductal delivery. When the part of the cut genome doesn't allow the animal to survive, DNA editing could be done more delicately, for instance, by inserting manipulated living cells. Besides investigating metabolic processes, the system allows following cancer developments and treatment. So far, animal models have been generated for rare and more common diseases. Among the more common diseases, besides cancer, are osteoporosis, Alzheimer's disease, and HIV-1/AIDS (14, 21).

The CRISPR technique is rapidly advancing. The DNA/RNA targeting Cas9 was soon followed by Cas12a, DNA, and Cas13a RNA targeting. Cas9 and Cas12a permit double-strand DNA (dsDNA) genome editing. Cas9 and Cas13a can be used for RNA alteration and to visualize DNA and RNA (15).

Indicating the substantial advancement of the discipline, at the end of last year an other examples of the Cas-system were published about an 'RNA-activate protein cleavage with a CRISPR-associated endopeptidase' from a bacterium called Desulfonema ishimotonii (22). For the expert, it is worthwhile to know that the system belongs to the Cas7-11 system with the TPR-CHAT protease Csx29. The bacterium used the mechanism to produce a toxic protein that inhibits the growth of the invading genome of the enemy but enables the scientist to work with RNA-guided functions 'in nature that can be leveraged for RNA-sensing applications in vitro and in human cells' (23, 24). TPR stands for 'true positive rate' and introduced a complex procedure to address a genome wide knockout CRISPR screen in a fly model (25).

'Screen' and functional genetic investigations

Screen is a more delicate approach for functional genetic investigations (CRISPR screening), making use of Cas9 modified cells. CRISPR screening often is applied to research genetic diseases with inherited pathological alleles, which means a version of the genetic sequence of a particular region of a chromosome ((14) (Figure 2D)). Lately, a first-in-human trial with patients who have Non-Hodgkin Lymphoma, a cancer caused by the overproduction of lymphatic system cells, was reported. The gene causing pathological T cells (Cart-T cells) were manipulated with a template based on gRNA derived from healthy donor T cells. Of six patients, four responded to the treatment within more than four months, one had a relapse, and one was still followed up at the time of reporting (26).

Further examples are clinical trials for sickle cell disease, beta-thalassemia, transthyretin (ATTR) amyloidosis (27), congenital eye disease, and progeria, the rapid aging in children. ATTR is caused by the misfolded transthyretin (TTR), which accumulates in the nerves and heart and is a deadly genetic disease. The therapy consists in producing through genetic engineering the substance 'NTLA-2001', which was generated to reduce the concentration of the misfolded TTR protein. The NTLA-2001, given through infusion, inhibiting the production of the faulty TTR in the liver (28). However, genetic editing is bound to fail. Recently a short notice informed that the inherited blindness disorder (Leber congenital amaurosis 10) was suspended since only three out of fourteen patients had had a vision improvement. The CRISPR treatment was injected into the eye (Science, Vol. 378, Issue 6622 (2022)).

'Prime editing' or the difficulty of hitting the target.

Therapeutic approaches in genetic diseases require accurate and precise editing. The DNA strand should be cut exactly where the cellular repair mechanism will be accomplished ((14) (Figure 3)). Recently 'prime editing' is expected to facilitate more precise editing, especially in avoiding 'random insertion of unattended targets' as expressed in the language of the scientists' (29). For gene insertions, the 'RNA guided DNA transcription' …' from co-delivered donor template into the target site' can be done ex vivo and in vivo. The ex vivo approach, for instance, is tried 'for editing hematopoietic stem cells and leucocytes.' The in vivo method injects CRISPR constituents of modified cells into the patients. The ex vivo method is limited to survival cells and can be cultured (16) (Figure 4). For instance, mice spermatogonial stem cells only survive when injected into newborn mice; a similar application cannot be applied to humans. The in vivo method faces the problem of bringing the editor into the organism. Lipid nanoparticles (LNP)

and a virus can be used as vehicles. Delivery can go wrong at many steps within the blood vessel and target cells. This consists of degradation, phagocytosis, being cached by the extracellular matrix, not being released from the vector, or didn't find the place in the DNA where the CRISPR process should evolve ((14) (Figure 3D)).

The failure is known as the 'off-target effect' and a major obstacle, particularly for CRISPR Cas9 for preclinical research and gene therapy and could account for more than 50% of failures. Critical are sequences of about twenty nucleotides of the sgRNA, which are complementary to the target DNA and followed by the PAM. The error can occur at the PAM distal part of the sequence. Different guide RNA configurations are prone to mismatch the on-target and off-target sites. The sequence crRNA, corresponding to the 3'end close to PAM, is also affected (30).

Both major vehicles, virus and LNP, have their serious drawbacks. Viruses as vectors can be dangerous, which is well known for the more orthodox development of vaccines (31) and for gene editing (16). The infamous mRNA products from Moderna and Pfizer, with numerous side effects such as allergy, and autoimmune diseases, could also be accounted to LNP vehicles used for the vaccines (32).

Ancestry problems with CRISPR.

Therapeutic improvements for cancer diseases with the help of genetic editing are one of the most interesting issues in clinical settings. There are cancer dependency maps for getting guide RNAs based on reference genomes. It was found that in two to five percent of the CRISPR approach in patients with 'Africa ancestry,' the CRISPR technique failed (33, 34). The reference genomes are derived from only a few USA citizens of Caucasian origin, so missing black USA citizens, for whom Caucasian references for CRISPR failed to work correctly. A similar problem might evolve when working with CRISPR for hemoglobinopathies in people of African origin (35).

Ancestry problems with CRISPR in the USA signal researchers on other continents, such as Asia, to be aware of genetic differences for different populations with different racial backgrounds. References from Western countries may not apply. A reference map for various races was introduced to solve the problem, which can be found as 'Ancestrygarden.org' (36).

Conclusion

No genetic editing scheme is approved by FDA as a standard therapeutic technique yet, except for mRNA vaccines. But governments shy away from admitting that mRNA vaccination has something to do with genetic engineering, and investigation into the damage done by mRNA vaccinations worldwide is suppressed by governments.

In 2018 He Jiankui 'innocently' reported at a meeting that he altered fetuses who, after birth, would be resistant to HIV. For changing the human genome to be heritable, he was sentenced to three years in prison. Other researchers should shy away from similar projects. Several serious problems are faced in theory, let alone in reality (37). In the USA, Congress banned research on

human embryos from being financed by government funds. Even if technical problems can be overcome and strict safety and sound quality are achieved, these therapies will be expensive (14). For countries with low resources, therapy gene editing daily will be challenging to finance.

A promising domain for improvements in gene therapy is cancer treatments. Besides that, other targets are disease patterns with acquired and inherited disorders. To date, there are 3.000 genes known to be associated with mutations causing diseases, and about 2600 gene therapy trials for disorders are on their way (38).

Fortunately, we are still far away from a biologically optimized spacefarer for intergenerational interstellar travel, living in Martian colonies as predicted by Cristopher E. Manson to be possible in the years 2251 to 2350 (39). And hopefully, the future predicted by a certain Mr. Harari in his book entitled '21 Lessons for the 21st Century,' who sees men's future in becoming God, will be as highly questionable as his understanding of history (40).

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