A Review of CRISPR with analysis and propositions of future applications

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Abstract

The etymology of CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats," which are periodically distributed at regular intervals, meaning a technique capable of 'recognizing' and editing a specific gene sequence, i.e. CRISPR

It's a combination of short gene sequences and CAS9, a protein that can find the desired sequence in billions of gene sequences and change it as we want. This CRISPR gene scissors is a technique commonly called gene therapy. With the release of genetic code specifying specific characteristics, scientists can insert or delete genes into the genome of virtually any plant or animal. In this paper, I explained the history and development of CRISPR. And proposed suggestions of utilizing it in various aspects.

Introduction

What is CRISPR?

Can human DNA be edited to cure various incurable diseases such as cancer, genetic disease, and degenerative disease? Since the emergence of a new "genetic scissors" called "CRISPR,"it became what is not completely impossible. CRISPR is a technology that changes genetic information of life. As a tool used to cut and repair the DNA of living organisms, it is widelyused not only for human DNA but also for repairing the DNA of plants, animals, and microorganisms.

The etymology of CRISPR refers to a technology that can 'recognize' and edit a specific sequence of genes as an abbreviation for “Clustered Regularly Interspaced Short Palindromic Repeats” that are periodically distributed at regular intervals. In other words, CRISPR is a combination of a short gene sequence and CAS9, a protein that can cut and attach DNA, which allows you to find the desired sequence in billions of genetic sequencesand change it as we want. This CRISPR gene scissors is a technology commonly referred toas gene therapy. With the disclosure of gene codes specifying specific characteristics, scientists can insert or delete genes into the genome virtually any plant or animal. (Here, the'genome' is genetic information that a creature has, commonly referred to as 'genome'). Thisprocess is simpler

and more effective than any other genetic manipulation technology. Scientists have now reasonably modified and manipulated gene codes that define all species on Earth, including humans, by modifying DNA in living cells.

CRISPR technology is an innovative technology that can correct DNA, the root cause of the disease, in the desired direction by being able to edit all DNA aggregates, including genes of living things, as if they were simple sentences.

Many scientists expect that CRISPR technology will completely change human life in the 21st century. This technology has opened a new era for mankind to dominate engineering and living things for just overnight.

The development of CRISPR

As mentioned above, genetic scissors are a method used to correct problematic genetic DNA,used to treat incurable viral diseases such as AIDS, genetic diseases such as hemophilia, andeven to prevent future food or environmental pollution.

The history of genetic scissors is not very long. It has been developed rapidly. Through the following article, we will examine the development of genetic scissors and the advantages and disadvantages of genetic scissors at each stage.

Genetic scissors technology is currently developed to the 4th generation, and CRISPR is the 3rd generation of them. The first-generation is "Zinc-fingernuclease (ZFNs, or called as Zinc-Finger), the second-generation is "Transcription Activator-like Effector Nuclease, TALEN", the third-generation is "CRISPR-Cas9", and the fourth-generation is "Scissor Prime Editor", discovered in 2019.

The first-generation genetic scissors are "Zinc-fingernuclease (ZFNs)." In 1985, scientists were studying African clawed frogs and discovered certain proteins attached to frog DNA. As a result of analyzing this structure, it was found that a material shaped like a finger and centered on zinc was firmly fixed in it, so it was named "ZFNs." The ZFNs protein adheres to the sequencing site of a specific gene and regulates gene operation. In particular, the nuclease linked to these ZFNs plays a role in cutting mutant genes that cause diseases. Nuclease refers to degradation enzymes (proteins) for nucleic acids such as DNA and RNA. As a feature, Fokl is used as a method of cutting DNA sequences, and the accuracy is only 24%.

The disadvantage is that the number of bases recognized by ZFNs is around 10 bases, and there is a high risk of cutting off unwanted areas when inserted into complex human genes. It is recognized as a lump, lacks precision, and new one should be made every time, so tt takes a lot of time and money. As a protein complex, there is also a concern that host cells may cause an immune response.

The second-generation genetic scissors are TALEN derived from vegetable pathogens. In 2009, scientists discovered a substance called TALEN in which proteins recognize bases. While ZFNs recognize multiple bases, TALEN can recognize bases one by one, enabling more sophisticated recognition. As a feature, Fokl is

used as a method of cutting DNA sequences, and the accuracy is up to 99%.

The disadvantage is that TALEN takes more than a month to make, is expensive, and is difficult to put into cells due to its large size.

In the end, the first and second generation genetic scissors had the disadvantage of having only about 10 gene sequences recognized and complicated production and utilization.

The first discovery of the third-generation gene scissors CRISPR was in 1987, when a team led by Dr. Soishino of Osaka University in Japan was studying the monoclastic gene of colon bacillus. This sequence was repeating Palindrome at regular intervals. Since then, after the first genetic map was released in 1994, the scientists who have looked at it find one common sequence. This was also a circular structure previously discovered at the University of Osaka in Japan in 1987. It is also found that there are 21 nucleotide sequences among these structures.

As a result, in 1995, it was discovered that there was a common palindrome sequence in several unrelated bacteria. This confirmed that CRISPR remembers certain substances. In 2012, Professor Daudna of the University of California, Berkeley, and a team of Ph.D. Sharfantier from Umeo University in Sweden discovered the enzyme which is CRISPR-Cas9 and introduced it in the paper.

CRISPR is an immune system originally created by bacteria to protect themselves from bacteriophages. Bacteria put phage's DNA in their genes and remember it, and when the same type of phage invades, they attack the virus using CAS9 protein enzyme. Scientists created a complex by attaching CRISPR and CAS9, which is the third generation of genetic

scissors, CRISPR CAS9.

The feature is that CAS9 enzyme is used as a method of cutting DNA sequences, and the accuracy is 90%.

The disadvantage is that the method of cutting the gene and injecting it back into the human body was mainly used, and it is also a problem to extract only the cells in question necessary for gene foundation from the human body. In addition, it is inevitable to consider practical problems such as injecting cells again after gene foundation to perform their original functions and extracting numerous cells that have occurred abnormalities one by one. The biggest problem is the transfer of a fairly large protein of CAS9 into the cell.

Despite the above disadvantages, CRISPR-Cas9 is easy to make and the production process is very short, about a day, so it costs less to make. In addition, various attempts are possible for accuracy higher than 90%, taking advantage of the short production process. So many scientists are currently preoccupied with research and development in this field to increase the accuracy and efficiency of this technology.

One of the surprising advantages of CRISPR Cas9 that we must know here is that ZFNs and TALEN recognize them as ZFNs proteins or TALEN proteins when recognizing bad genes, but CRISPR can use RNA to find DNA sequences

that fit them exactly like a guided missile. This may be due to the structure consisting of a guide RNA in which CRISPR CAS9 binds to a mutant gene and a CAS9 nuclease protein that cuts off the gene site. In addition, if the guide RNA is replaced with another RNA as needed, genetic scissors for the treatment of new genetic diseases can be made as much as possible, so the range of use is very high indeed.

CRISPR can correct the genes in the cells taken out of the human body and then put them back into the body, or enter the body directly to treat the genes. How accurately can we return treated or corrected DNA to our bodies in the future? The problem of finding a way can be seen as an important clue that will determine the success of this technology.

The 4th Genetic Scissors Prime Editor

It was developed by a team of professors David R, Liu of Harvard University. On October 21, 2019, 75,000 mutations identified in the human genome in Nature are known to be the cause of genetic diseases, of which genetic scissors expected to be able to treat about 89 percent were introduced.

The previous scissors, including the third-generation genetic scissors, were excellent in erasing DNA codes in certain areas to remove unwanted gene functioning. The big advantage of the breakthrough was that it accurately found and removed unwanted points. However, there was no function to modify the sequence of DNA changed by mutation. Genetic diseases that could be treated depending on the ability to erase gene codes were bound to be limited to those that revealed in tissues where too many or should not have been revealed.

Prime Editor, the newly developed fourth-generation genetic scissors, not only removes specific sequences as researchers want, but also allows other sequences to be inserted into the place. The name of the new technology is also similar to using a word processor to erase words and put new words in the place, so it is sometimes called the "best braces". Also it has become ‘Prime Editor’, since this has the meaning of the starting point of something at the same time.

The advantage of the Prime Editor is that in the case of CRISPR CAS9, the position to be deformed must be close to the PAM sheet, but the Prime Editor does not matter if it is separated from the PAM to 30 nucleic acids. And the Prime Editor has the advantage of being able to change the base precisely as desired without any problems. If homologous recombination is used in the area to be corrected with CAS9, the efficiency is about 10%, and there is a problem that many by-products are cut and attached in different lengths. In addition, CRISPR CAS9, the third-generation genetic scissors, has a problem of transforming areas other than targets, and Prime editor does not produce by-products and have the advantage of correcting them to only one place they want.

The disadvantage of Prime Editor is that at this stage, the means to put fourth-generation genetic

scissors in patients has not been developed, and although new driver scissors have many advantages, they are not yet an all-around technology to replace completely the third-generation genetic scissors, CRISPER-CAS9. And CRISPR stores information necessary for modification in DNA, while Prime Editor stores it in RNA. RNA has its own limitations because it is more susceptible to damage and the longer it is, the higher the possibility of damage. Therefore, the fourth-generation genetic scissors are suitable for modifying short sequences and are not suitable for modifying or replacing the entire long gene.

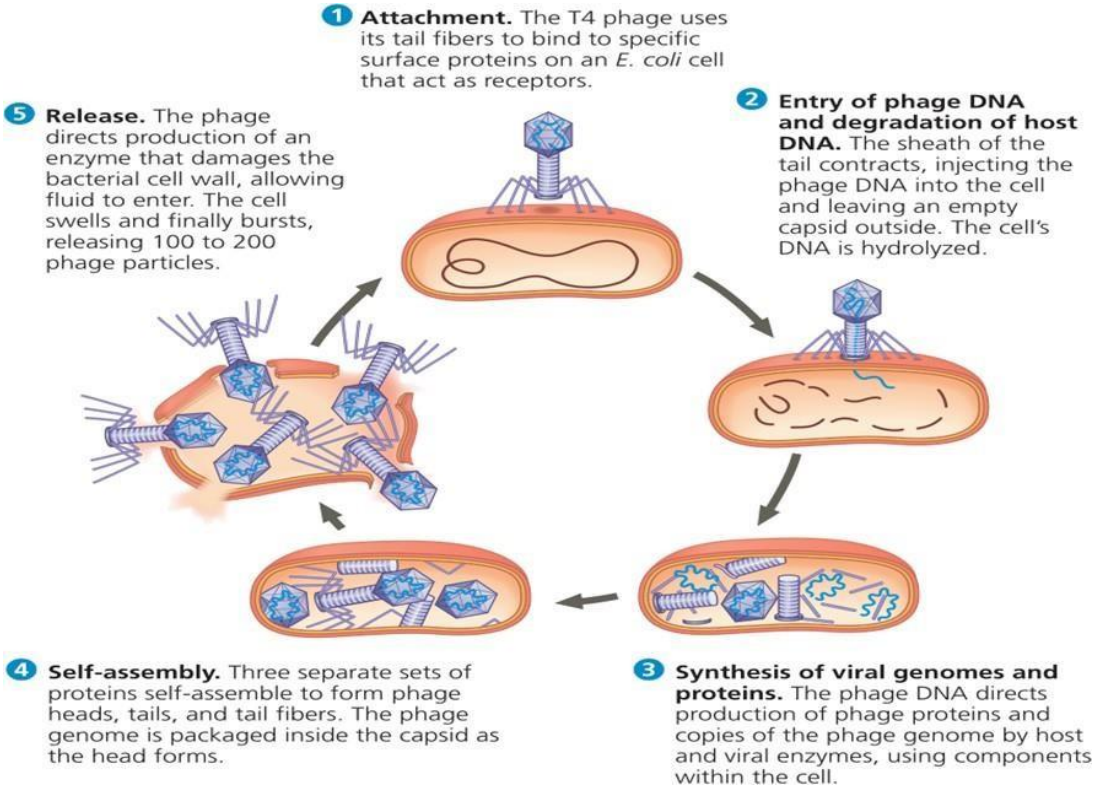
Main Points

The mechanism of CRISPR

Just as humans create memory cells to prepare for secondary immunity when infected with the virus, it was found that the bacteria are responsible for obtaining immunity from genetic substances introduced from outside to protect themselves from virus attacks. Bacteriophage, a virus that is infects bacteria, cannot replicate itself outside of cells. So, they enter the host cell, replicate themselves using the metabolic system in the cell, and then the cell is killed. In other words, Bacteriophage binds to the receptor of bacteria, injects its own DNA into the host cell to hydrolyze the host cell's DNA, synthesize its own DNA and protein using the host cell's enzyme system, and re-synthesize its body in the host cell. Bacteria phage destroys the cell walls of bacteria using the generated enzymes, and as cells swell, 100 to 200 particles of bacterial phage burst out of the cells and are released.

In this way, host cells are infected and killed by bacteriophage. However, bacteria also operate the defensive immune system to protect themselves, which is the action of CRISPR.

The principle of action of CRISPR is that when

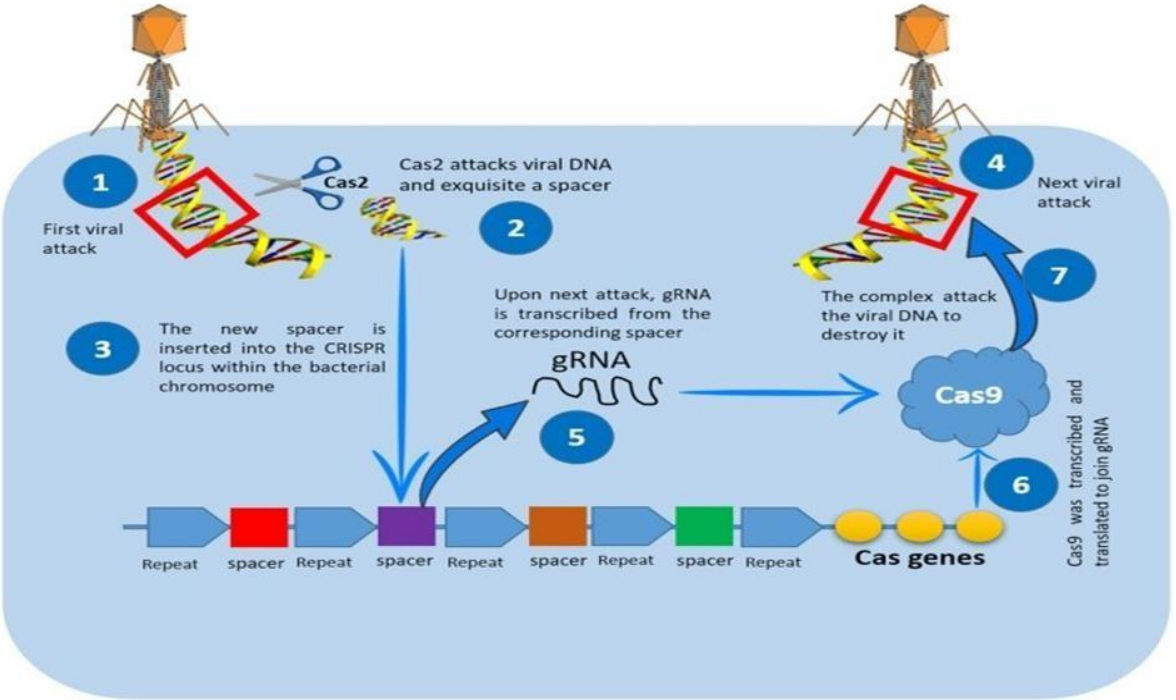


bacteriophages invade bacteria, the CAS protein acquires a proto-space from bacteriophage DNA and stores the DNA of these bacteriophages in interspaces (32 to 38 base pairs) between DNA bases with Paliendrome structures in our body. Gene expression occurs in the DNA part of the stored bacteria, resulting in mRNA. This is the guide RNA (CRISPR guide RNA, crgRNA). The RNA

created in this way holds the genetic code of the invading virus intact. Using this information, when the same type of virus invades, it complementarily binds the virus DNA to a specific base sequence in the virus' DNA. Genes are expressed in the DNA Cas gene site of the bacteria bound in this way to form Cas proteins. Cas stands for the CRISPR Associated System and can be considered a protein complex associated with CRISPR. The protein mass, the main component of the enzyme, is CAS9. Bacteria send guide RNA to cut off the virus

DNA that has invaded our body so that they can hold tight to the target sequence of the virus that has invaded, and CAS9 binds to guide RNA to cut off the virus' DNA. The virus whose DNA is cut off by CAS9 eventually dies without infecting the bacteria. To easily explain the principle of action of CRISPR, a bacterial immune system, the virus DNA invading the bacterial adaptive immune system is fragmented, stored in its own specific nucleotide sequence, and when the same virus enters again, the guide RNA sticks to the virus's DNA and blocks the virus's infection and proliferation.

In the scientific community, which has deciphered certain human gene sequences since 2003, customized guided RNA (guide RNA) that can complementarily bind to the gene through genetic scissors and insert the appropriate CAS9 enzyme to prevent the expression of the gene that causes the disease. In addition, this opened the way for treatment such as incurable diseases and incurable genetic diseases. Also, with the discovery of CRISPR CAS9, in the case of



animals and plants, we can improve the traits we want to make what we need

Examples of CRISPR technology

Application to treat human diseases

CRISPR-CAS9 can be used to treat incurable AIDS and cancer. The method is to treat diseases by collecting immune cells from the patient's body, modifying the genes of these immune cells, and injecting them again. AIDS is caused by the infection of immune cells with human immunodeficiency virus (HIV), and people who do not have the gene "CCR5" by nature do not get AIDS. Researchers at Sangamo biosciences, a U.S. biotechnology company, had removed the "CCR5" gene from immune cells of AIDS patients and administered it back into the body, and then it was found that both the reproduction of HIV and the storage where HIV disappeared in the patient's body decreased. Academia already believes that CRISPR can cure AIDS.

CRISPR is also attracting attention as a new weapon to conquer cancer. In June 2020, the National Institutes of Health (NIH) approved a CRISPR clinical trial for cancer treatment by researchers at the University of Pennsylvania in the United States. It is a treatment method that corrects the genes of immune cells extracted from cancer patients' blood with genetic scissors and injects them back to attack cancer cells.

In July, Dr. Luyu's team at Sichuan University in China was approved for a clinical trial plan in the body after extracting T cells in the blood from lung cancer patients, removing target genes using CRISPR.

In addition, scientists at Johns Hopkins Medical School in the United States have published research that could be an important clue to blocking metastatic cancer. Metastatic cancer refers to the transfer of "Cyclic Tumor Cell Cluster" (CTCs), a group of cancer cells originally separated from the cancer site, to other organs or tissues through blood. In fact, it is no exaggeration to say that most cancer deaths stem from metastatic cancer. However, the study found on July 7, 2021, that a protein called "TRPM7," known to be involved in calcium control of cells, can prevent blood flow into a group of cancer cells that have deviated from the original cancer, in a paper published in the journal "Science Advances." According to an overview of the paper released on the American Science Promotion Association (www.eurekalert.org) on the 14th, the "TRPM7" protein detects the pressure of flowing blood and prevents cells from spreading to blood vessels. Metastatic cancer cells have very low "TRPM7" levels, making it easy to enter blood vessels, and the research team confirmed that they can prevent vascular entry and metastasis by increasing the expression of "TRPM7" in tumor cells. The research team expects that the discovery could lead to the development of cancer treatments that regulate the activity of related genes with CRISPR scissors.

CRISPR-CAS9 can also be used to treat diseases caused by genetic mutations. In June 2021, The Science published the results of a clinical trial that used CRISPR to deactivate the Transcyretin (TTR) protein mutation gene. When a drug

equipped with CRISPR system was injected into the blood of patients suffering from TRANSTHYRETIN AMYLOIDOSIS caused by genetic mutations in the transstyretin plasma protein, abnormal protein production was almost stopped and no side effects appeared. An effective treatment for directly injecting CRISPR-CAS9 systems targeting proteins produced in the liver was developed by Intella Theraputics, co-founded by Jennifer Dodna. Most human genetic diseases cannot be taken out of the body, so they should be treated using a method of direct correction in the body.

And CRISPR-CAS9 can also be used as a treatment for genetic diseases. Hemophilia, which it does not stop once bleeding due to a lack of protein that strengthens blood in the blood due to genetic mutations, is a typical genetic disease. Hemophilia is a disease caused by a genetic mutation that causes certain parts of genes to be overturned, and domestic researchers have succeeded in collecting cells from the urine of hemophilia patients to make reverse differentiated stem cells (IPS cells) and correcting overturned genes using CRISPR gene scissors. As a result of differentiating normally corrected stem cells into vascular endothelial cells that make blood coagulation factors and transplanting them into "hemophilia" mice, it was confirmed that blood coagulation factors were produced in mice, significantly improving bleeding symptoms.

A project to restore extinct animals.

Currently, the projects to restore extinct animals are in full swing throughout the Earth. In particular, the recent rapid development of gene editing technologies such as adding or subtracting certain genes, such as CRISPR-Cass9, is also raising the possibility of restoration projects.

"Tasmanian wolf ", once the top predator of the Australian continent and Tasmanian island, was indiscriminately over-captured by herdsmen for attacking sheep, and eventually killed even the last pair raised in the zoo, resulting in the extinction of the Tasmanian wolf in 1936.

Professor Andrew Pask of Melbourne, Australia, and his research team are challenging the restoration of the Australian Tasmanian "Tasmanian wolf." In December 2017, they published the results of DNA collection and analysis from a 105-year-old sample of Tasmanian wolf cubs in Nature magazine. This is the first case in which genetic maps of extinct wild animals have been analyzed, and they are conducting research to synthesize proteins and enzymes, just like the analyzed Tasmanian wolf gene map, to create a new Tasmanian wolf using Tasmanian wolf as a surrogate. This is a method of comparing and analyzing the genes of an extinct animal and its descendants to find the genetically closest one to the extinct species, and inserting some genes that determine the genetic characteristics of the extinct species through CRISPR genetic scissors into the descendants' genes.

In March 2018, a team led by Professor George Church of Harvard University, a developer of CRISPR-Cass9, announced that they would restore the "Mammoth "that went extinct 30,000 years ago in the same way. The team announced that it would restore the mammoth by attaching characteristics such as heaping hair and

additional subcutaneous fat that can survive the cold, a genetic characteristic of the mammoth, to the genes of "Asian Elephants" genetically similar to mammoth. The theory is that some of the genes of Asian elephants are converted into the genes of mammoths using CRISPR to create adult elephants with long hairs on small ears and characteristics of mammoths with a lot of body fat, completing mammoths.

Application for future food development

Controversy over the harmfulness of Genetically Modified Organisms (GMOs) is still ongoing. Most of the GMOs currently being made use of "agrobacterium," a plant bacterium, to artificially insert external genes into plants. In this process, hundreds or thousands of external genes are inserted, which can lead to safety problems. In other words, GMO is based on genetic modification, but CRISPR can be free from the stability problem of GMO due to the substitution of the original gene, not genetic modification.

CRISPR technology can be used to reduce crop losses - In a recent review of January 1, 2021, the international horticultural journal "Horticultural Research" predicted that gene editing technology using CRISPR-CAS9 could reduce crop losses and waste within five years.

The World Food and Agriculture Organization designated September 29, 2020 as 'International Food Loss and Waste' Recognition Day and said, "More than 14% of the world's food is lost or discarded." Horticultural crops disposed of as garbage account for 33% of all agricultural products due to their short shelf life. This loss of crops can cause another environmental problem, including waste of packaging and greenhouse gas emissions. So preventing losses after harvesting has become a research task for many scientists.

In order to prevent the plant disposal, ethylene control, the main culprit of crop aging and aging, is the key. According to the paper, it is easy to control the ethylene process, the main culprit of crop aging, by cutting or inducing substitution of transcription factors involved in ethylene biosynthesis in plants with gene editing technology. For example, controlling the "ethylene synthesis" of plants allows us to freely control the aging period of fruits such as tomatoes, bell peppers, oranges, potatoes, and root vegetables. In addition, editing the key genes that break down the fruit epidermis cuticle layer and cell wall can increase resistance to fungal infection. In addition, anthocyanin, which is responsible for the color of crops, sugar and starch, which are responsible for taste, and browning can be improved through gene editing.

If this improvement brings a better appearance and taste of crops, agricultural products thrown away after harvest will be significantly reduced. In addition, the abnormal climate in countries around the world due to global warming will have a great impact on our future food supply. Despite these environmental changes, genetic scissors technology is expected to be worth protecting human life in the future.

In December 2020, genetically modified pigs were approved for use by the U.S. Food and Drug Administration (FDA) for food and medicine.

The FDA said "GalSafe" is the first "genetic modified animal" approved simultaneously for "eating" and "medicine," and a sugar component called Alpha-gal, which causes allergies to the surface of pig cells, was removed by genetic manipulation. People allergic to Alpha-gal have symptoms such as hives, itching, convulsions, and vomiting when eating beef, pork, and lamb, and these symptoms are known to occur in certain tick bites. Genetically modified pigs were developed by United Therapeutics, a U.S. biotechnology company, under the name "GalSafe," and revealed the reason for the development of "GalSafe," saying, "We are trying to create organs that can be transplanted into the human body in the long term." This is the second FDA approval after Salmon in 2015, and it can be said to be a tremendous milestone for scientific innovation in that it is the first approval of food and pharmaceutical animal biotechnology products.

In addition to the development of GalSafe pigs, gene scissors can be seen as an example of producing pigs with good meat quality by removing genes that inhibit muscle growth from pig embryonic cells and then transplanting them into egg cells.

Apart from that, genetic scissors technology can have the effect of preventing pests by cutting genes that help infection from wheat DNA vulnerable to pests, and can develop bananas disease resistant to pests by removing fungal receptor genes that cause banana diseases. As such, the use of genetic scissors technology is endless and can be said to be an area that needs to be further researched and developed in the future.

CRISPR's market size

This technology is currently attracting more attention for the development of CRISPR-based diagnostic tests to solve sample collection and timeline problems for COVID-19 testing. The reason is that they can utilize advantages such as higher accuracy, sensitivity, fast detection, and portability of the test object, if CRISPR- based tests are activated.

CRISPR gene editing technology is creating a new trend not only in medicine but also in the food industry. In April 2016, the U.S. Department of Agriculture (USDA) decided to differentiate discoloration prevention mushrooms made with genetic scissors technology for the first time in the world from genetic manipulation technology, not a subject to GMO safety regulations.

In the case of the Japanese government, the 'Integrated Innovation Strategy' was resolved in June 2019 and announced that “Not GMO technology, but food that maintains its own genes and cuts and improves parts of DNA" which causes genetic modification by inserting genes, would be excluded from safety regulations. Only information on which genes have been removed from foods improved with genetic scissors needs to be disclosed to the government and consumers. As such, the field of use of genetic scissors is gradually expanding. As a result, the competition between major developing countries and global companies, including patent disputes

to preoccupy this market, is fierce.

According to a report by Grandview Research on May 14, 2021, the size of the gene market was estimated at about $1.5 billion in 2020, and the average annual growth rate (CAGR) is expected to be 21.7% from 2021 to 2028.

As of 2020, CRISPR-based products accounted for 79.4% of the total sales of the "Genetics Editing", and sales in the field are expected to rise further in the future as many researchers participate in CRISPR technology development.

It is no exaggeration to say that CRISPR technology dominated the global overall market in the field of biomedical application in 2020. 92.8% of sales came from the biomedical application sector.

Users of CRISPR technology were Biotech and major pharmaceutical companies, accounting for 48.8% of End user's income.

Looking at the regional share of the CRISPR market, North America, which publishes the most research on CRISPR, accounted for 38.4% of CRISPR's total sales, and is expected to grow rapidly in the Asia-Pacific region in the future.

Editas Medison, founded by the Broad Research Institute, was the first CRISPR company to be listed on NASDAQ, and received $120 million in investment from a research fund called Vengo and Google Ventures founded by Bill Gates.

Intellia Therfusc, founded by Professor Dowd, who won the Nobel Prize in Chemistry, was also listed on NASDAQ and received $15 million in investment from Novatinova. In addition, the Swiss bio-venture CRISPR Therapystic, co-winner of the Nobel Prize in Chemistry, where Dr. Sharfantier is in charge of technology and torture, was jointly established with Bayer, Germany, and receives $330 million in support.

In Korea, ToolGen, which has CRISPR gene scissors technology developed by Kim Jin-soo, head of the IBS Genetic Correction Research Group, is on par with global companies.

Advantages and problems of CRISPR

CRISPR is considered the "best scientific achievement" selected by the two major scientific journals such as "Nature" and "Science" in 2015, and was selected as one of the top 10 technologies of MIT Technology Reviews in 2016. In the end, this breakthrough technology gave the Nobel Prize in Chemistry to Professor Jennifer Daudna of the University of California, Berkeley, who developed the third generation CRISPR-CAS9, and Emmanuel Sharfantier of the Maxplanck Institute in Germany. Professor Downa expects the technology to be widely used for human health and welfare as a CRISPR developer, but is concerned about the abuse of technology to improve human appearance, intelligence, and athletic ability in the near future by dictators such as Hitler or selfish parents.

The problem of CRISPR genetic scissors technology has been steadily discussed not only among "life scientists" but also among forensic and ethical scholars since its development. For example, in 2017, a paper published in Nature magazine stating that "MYBPC3, a mutant gene

that causes incurable diseases called "hypertrophic cardiomyopathy," can be removed from embryos through "CRISPR gene scissors

CRISPR-CAS9" technology.

Major media outlets around the world made headlines about Oregon Health & Science University (OHSH), which conducted the study, saying, "It is the result of the study that approached the clinical stage that can give birth to a healthy child." On April 1, 2018, Dr. Hwang Junju's team at China's Zhongshan University announced the result of editing the human fertilized egg genome using CRISPR. Although it was not a significant achievement due to its low efficiency, it received great attention as it was the first case of applying CRISPR to humans. And on November 27, Professor He Jiankui, a Chinese scientist working at the University of Southern Science and Technology in Shenzhen, China, made a bomb statement that "we tried to edit genes using the 'CRISPR-CAS9' technology for the embryos of the seven couples who were undergoing pregnancy promotion treatment, and one of them gave birth to twins." Professor He Jiankui said, "I feel a great responsibility for performing the world's first gene editing procedure on a baby who will be born," and requested, "The international community should decide whether to allow gene editing procedures to happen in the future."

Professor Kiran Musunuru, a geneticist at the University of Pennsylvania, expressed strong anger, saying, "Genetic editing technology for babies is ethically unacceptable," and Professor Julian Savulescu, a practical ethicist at Oxford, "It can lead to unexpected mutations and more cancerous life."

A special article of The Wire concerned that the birth of a baby artificially edited with genes could cause more serious situations than the competition for nuclear weapons development in the past or current weather conditions. This is because replacing a human gene means that all cells of a baby to be born in the future can be replaced as needed. Depending on how this technology is used, it can have terrifying consequences that can cause another disaster for mankind. If so, it may be necessary to find out more about the problems of genetic scissors and study together how to develop this technology in the future.

The first problem is the safety issue of CRISPR-CAS9. The most controversial issue in commercializing CRISPR gene technology may be this safety issue. The accuracy of CRISPR-CAS9 remains at the 90% level, not 100%. This 10% error can cause a bigger problem than we think. And there is still no way to measure whether genes in the desired area can be accurately removed when using genetic scissors. There is a risk that CRISPR gene scissors will mutate in the wrong gene sequence similar to the base sequence of the target gene, and it cannot be ruled out that mutations may occur during gene editing, resulting in unexpected side effects. Especially the verification of the off-target effect problem, which causes unintended mutations outside the target gene, is a very important part to

be dealt with. Some argue that technology to reduce target deviation has been sufficiently developed, but some scientists argue that more discussions are needed on the acceptable level of target deviation for safety. Currently, CRISPR's research and development process is short, so it does not have enough data to prove the stability of the results.

A study in 2018 by Professor Matthew Fortus of Stanford University suggested that mutations in genes, deletion, replication, and other changes in genes can cause many diseases.

The second problem is ethical issues. Genetic treatment methods include somatic cell therapy and reproductive cell therapy, and treatment methods that can cause ethical problems can occur in reproductive cell therapy. Compared to somatic cell therapy, which does not affect descendants and is limited to those treated, reproductive cell therapy changes the genes of the embryo by treatment in the early stages of eggs or sperm, spouse cells or humans, and this change is transferred to the next generation to become genetic.

The problem of intentionally modifying genes to human embryos includes philosophical problems beyond ethical problems. If human embryos are regarded as a life form, this could be a human invasion of the realm of God. In addition, the use of genetic scissors technology for human embryos is not guaranteed safety, moreover there is a concern that human needs will deal with embryos for unethical purposes. If some privileged people can examine genes from the early stages of embryos and modify them through genetic scissors when problems arise, there will be a risk that this will spread beyond ethical problems to socioeconomic problems such as social inequality and widening status gap.

Even Jennifer Dowd, a professor at UC Berkeley who identified the editing mechanism of CRISPR-CAS9, points out that gene therapy for human embryos is necessary, but stability is still not secured. He insists that Genetic scissors technology can be widely used to prevent, treat diseases, and develop agricultural and livestock products, but there must be a guideline for the scope of use are needed due to its great ripple effect.

As the ripple effect of genetic scissors is increasing socially, it seems necessary to discuss with specialists from all walks of life how to use it in the future. I presume that it would be good to show a responsible attitude by actively participating in social discussions on technology as well as research in the laboratory.

Conclusion

Future applications of CRISPR technology

CRISPR gene scissors are a technology that recognizes a specific base sequence in the genome of animals, plants and cuts or corrects DNA. This technology can correct and improve genetic information of living things as almighty.

Currently, this technology is contributing greatly to mankind, such as research and development of crops to treat genetic diseases and increase food. In the future, it is expected that this technology

can be used greatly in developing livestock or grains that can adapt to changing environments from time to time due to abnormal climates.

In the medical community, researches on rapid and accurate molecular diagnosis with the COVID-19 virus are being actively conducted these days. The diagnostic technology developed by Professor Downa of the University of California, Berkeley, who won the 2020 Nobel Prize in Chemistry, released on the paper's pre-public site MedArcive, is a very innovative technology that quickly and accurately determined COVID-19 positive without gene amplifiers or expensive equipment. With this opportunity, it is expected that studies that can quickly and accurately diagnose many infectious diseases using CRISPR will become more active. Professor Downa predictaed that in addition to these diagnostic technologies, technologies for producing various new drugs using genetic engineering yeast could be developed within a few years.

I thought about “why don't I study how to treat human aging using CRISPR genetic scissors?” Currently, CRISPR is used to treat genetic diseases and cancer incurable diseases, but all of these diseases are actually deeply related to aging in our body. Dr. David Sinclair, a professor of genetics at Harvard Medical School, argues in the book titled The End of Aging that "aging is only a matter of disease that can be cured."

If you regard aging as a disease and think of it as a treatment object, like Dr. David Sinclair, I think it is necessary to find out what the genetic difference is between the long-lived and people who do not.

In Japan, blood tests were conducted on 140 elderly people aged 110 or older by selecting 7 healthy men and women. Their blood showed more "killer T cells," immune cells that attack cancer cells, etc., than ordinary people. In particular, special immune cells called "CD4 positive killer T cells" were found to be 10 times more than the average of 45 other experimental groups aged 20 to 70 years old. In the end, it was found that those who live along life have higher immunity to infectious diseases and cancer than the general public.

According to international researchers in Italy and the UK, people aged 105 or older are likely to have special genes that increase the efficiency of their ability to recover DNA.

Professor Paolo Garagnani of the University of Bologna, who led the study, recruited a total of 81 super-aged people aged 105 or older and 110 or older, and later collected blood samples of 36 healthy elderly people aged 68 to confirm the genetic difference between the super-aged and the elderly. As a result, it was found that changes in certain genes related to DNA recovery, cell health status, and self-destruction of damaged cells were common in super-aged people over 105.

The researchers identified five common genetic changes between the two genes calle "COA1"(Gene that produces cell energy and has the key to maintaining proper communication between mitochondria and cell nuclei, where dysfunction is a key factor in aging) and "STK17A"(Gene involved in excessive cell

response to DNA damage, dangerous active oxygen levels, and self-destruction of damaged cells), which are more frequently present in groups of "105 to 110 years old" and "120 years old or older." It also found that gene modification is common in older people over the age of 100.

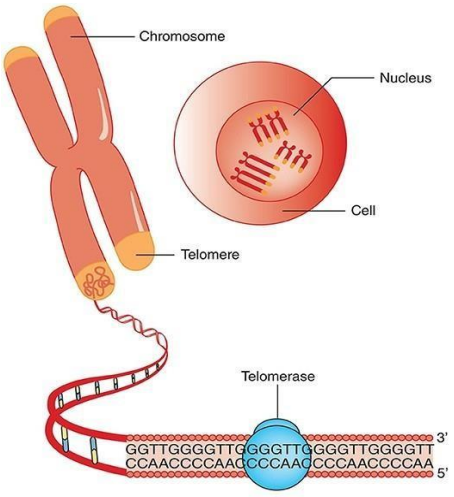
Through the above two experiments, we were able to identify the characteristics of DNA in healthy long-lived people, and hypothesized that it is possible to edit both genes "COA1" and "STK17A" with CRISPR genetic scissors to enable DNA recovery.

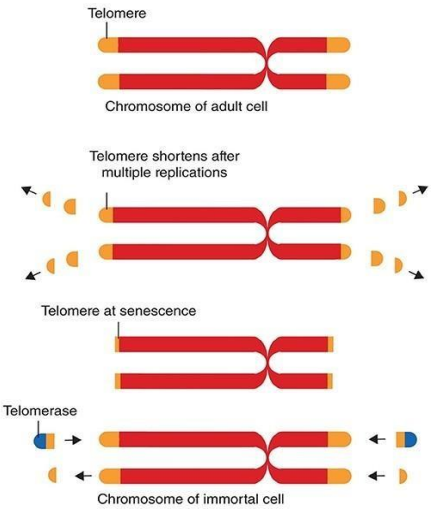
Dr. Christina Giuliani, who participated in the study, said, "DNA recovery is one of the mechanisms that extend lifespan across multiple animal species, and this is the result of experiments that confirmed that it is also true for humans." This experiment is the first case of elaborately detoxifying the genes of people living long lives, and is meaningful in that it revealed how some people can avoid the horrors of age-related diseases even though they live long. (International journal eife, May 2021)

The biggest advantage of genetic scissors lies in the technique of accurately cutting and editing the problematic part. Then, on the contrary, if we find DNA that causes aging and cut off only this part, wouldn't it be closer to how to treat aging? To find out about this, we needed to find out about people with aging genes.

Dr. Nilesh Samani, a cardiologist at Leicester University in the UK, reported on January 7, 2021 in the British daily Guardian Internet edition that people with these mutations biologically age three to seven years earlier. Dr. Samani analyzed about 500,000 DNA sequencing mutations in the gene in about 3,000 people and found that those who mutated the DNA sequence right next to the gene called TERC in chromosome 3 increased faster than those who did not, and suffered from senile diseases such as heart disease. 38% of them inherited only one copy of the mutant in question, and they age biologically three to four years faster than those with normal genes, and those who inherited both mutant copies aged six to seven years faster.

According to Dr. Samani, people with mutant genes have abnormally short telomeres of chromosomes for their age. Here, telomere refers to the wrapped protecting part like plastic so that





the shoelaces do not loosen at both ends of the human chromosome.

The telomeres are gradually shortened in length for each time the cells divide, and later become significantly shorter and disappear. Then the cell loses its function and sends a signal of aging. The gene called TERC is a gene that produces telomerase, a reverse transcription enzyme that protects telomeres.

Dr. Samani speculated that those with one or two mutated gene copies would have made less telomerase since they grew up in the womb. In the end, they were born with short telomeres from birth, so they have no choice but to grow old quickly. Academia injected insufficient telomerase for treatment. It warns that there is a risk of causing cancer instead of preventing thin aging. This is because telomerase is completely deactivated after human birth, but as it is reactivated in cancer cells, cells do not age and proliferate indefinitely. If there is a way to correct mutant genes without injecting telomerase into the human body, wouldn't aging genes be removed without side effects? If the mutant gene can be edited with CRISPR gene scissors and returned to normal in the human reproductive cell or embryonic cell stage, it may be possible to extend their sleep of three to seven years. The findings were published in the latest issue of Nature Genetics on February 7, 2021.

For scientists, a research on human aging is quite attractive and has many tasks to solve in the future. In fact, recently, research data that has made great achievements in the treatment of aging-related diseases using genetic scissors have been reported one after another.

In fact, there is an example of extending the lifespan by treating progeria with gene editing technology. The study, published on January 6, 2021, in the scientific journal Nature, succeeded in prolonging sleep in mice with congenital progeria using the latest DNA editing technology. Congenital progeria is a genetic disease that causes extreme early aging in children and greatly shortens human lifespan. Participating research teams were jointly conducted by the National Human Genetic Research Institute (NHGRI) under the National Institutes of Health (NIH), the Harvard MIT Broad Research Institute, and Vanderbilt University Medical Center.

DNA, a human genetic material, consists of four circular bases: A, C, G, and T. Congenital progeria, also known as Hutchinson-Gilford Zoro syndrome, is known to be caused by a mutation in which one DNA base C changes to T in the nucleolamine A protein (LMNA) gene that makes up the nuclear lamina of cells. This increases the production of PROGERIN, a toxic protein, and promotes aging rapidly. In general, it is estimated that about one in four million children is diagnosed with morbidity within two years of birth.

These children actually show symptoms such as cardiovascular diseases such as heart attacks and strokes that the elderly suffer from in childhood and adolescence, and hair loss, skeletal problems, subcutaneous fat loss, and skin hardening.

This study used a groundbreaking DNA editing

technique called Base editing, which replaces single DNA characters with other characters without damaging DNA.

This base editing method was developed in 2016 by Dr. David Liu's team at Broad Laboratory with funding from the Human Genome Research Institute.

Dr. Liu, one of the senior authors of the paper, points out that "CRISPR editors are innovative, but DNA cannot be accurately changed in various types of cells yet."

However, he said, "The base-editing technology developed by the Ph.D. team is similar to the search-and-change function of the document-written word processor, so it is very efficient in converting one base pair into another, so we expect it to have a strong effect on treating diseases such as progeria."

In order to test the effectiveness of the base-editing method, the research team was able to work with The Progeria Research Foundation to secure binding tissue cells in patients with dysmenorrhea and to convert the nucleolamine A protein (LMNA) gene base in patients' cells.

Following success, the research team used this technology to explore how these mutation changes affect symptoms such as progeria in mice.

Immediately after birth, DNA editing mixtures were injected into veins once into about 12 mice with morbidity-causing mutations to successfully restore normal DNA sequences of LMNA genes in significant proportions of cells in various organs, including the heart and aorta.

The results were much better than expected because numerous rat cells tested still maintained a corrected DNA sequence six months after treatment, and edited cells in the aorta appeared to have been out of early deterioration by replacing cells with "progeria" mutations. The lifespan of mice used in the study is two years, and the fact that the lifespan of mice treated has doubled from seven months to 1.5 years is a very valuable study result. Dr. Francis Collins, a senior researcher at the Human Genetic Research Institute and director of the National Institute of Health, said, "we were able to have a belief that we could come up with a tool to cure the root cause of the disease in the fact that this disease occurs in almost all children affected by a single mutation."

The study is said to have set another milestone for research on progeria following the U.S. Food and Drug Administration's approval of the first treatment for progeria called lonafarnid in November 2020. In the case of drug therapy, life expectancy can be extended for some time, but while DNA editing is not a complete treatment, it is expected to provide dramatic treatment options that can expand treatment options in the future.

Human aging has been shown to affect not only the inside and outside of the body but also the function of the brain. Aging is the main biological factor that causes neurodegenerative diseases such as Alzheimer's. A research team at Columbia University's medical school published in the journal Cell System reported that certain genes in brain areas responsible for high-quality cognitive

functions such as human reasoning and memory can accelerate brain aging and cause degenerative diseases such as dementia.

The research team examined and analyzed 1,904 brain tissues that did not suffer from neurodegenerative diseases. Each tissue was divided by age through technology to estimate brain age with molecules in the brain. Then, compared to other genes that normally develop over time and progress aging, the tissues of each age group were identified to accelerate brain aging.

As a result, researchers found that a gene called "TMEM106B" is related to the aging rate of the brain. Among these genes, elderly people aged 65 or older with two sets of specific mutations had an average aging rate of about 12 years faster than that of ordinary people. TMEM106B is turned off until about 65 years old and then turned on after 65. The research team warned, "Neurodegenerative diseases can look healthy when they are young, but they can start at a certain age." People with the gene mutation may be vulnerable to brain-related diseases due to accelerated brain aging. If the "TMEM106B" gene, which is the cause of aging in the brain, can't it be edited and modified using CRISPR gene scissors to return to normal, it can prevent and prevent anxiety factors that may cause dementia or cognitive decline by aging the normal brain?

The causes of aging are so diverse that we are not aware of them all. But if we can solve this homework, we will meet a new level of world.

Conclusion

The remarkable economic and science development have given us such a rich world.

However, in return for that, abnormal climate problems caused by global warming and the emergence of various unexpected viruses have come to our side very quietly. Currently, global warming causes floods or fires in each country, causing great physical and economic losses, and the biggest problem with this phenomenon is that it does not end up as a temporary problem, but as the beginning of future problems. In addition, a virus called COVID-19, which has emerged since the end of 2019, has closed the doors of each country, and the resulting mental and physical losses have reached a point where it.

The scientists believe that given all these phenomena, we should be prepared for all the problems that we can imagine today. So, in this point, CRISPR gene editing technology could be a breakthrough solution that can solve these various problems.

Currently, scientists are banning research using CRISPR technology in human reproductive cells or embryonic cells in consideration of ethical issues. However, considering the rapidly changing surrounding environment, humans may someday be in a situation where this gene editing technology becomes a necessity, not an option, to survive. Those who study science should develop it with an open mind, keeping in mind all the possibilities that will happen in the future. I think the more students who have this idea of science, the greater the hope of solving the problems that are facing us.

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