Symposium

From Bench to Bedside: The Evolution of Human Genome Editing in Clinical Practice

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Abstract

Genome-editing is a precise technology used to insert, delete, replace or modify a specific DNA sequence, and also used for epigenome and gene regulation. The haploid human genome (23 chromosomes) is about 3 billion base-pairs long and contains 20,000–25,000 distinct protein-coding genes. Genome-editing tools include meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) systems. Genome-editing technology is mostly used in research and ongoing clinical trials to diagnose and treat certain conditions including hematologic disorders, cancers and infectious diseases. There are some technical challenges, safety and bioethical concerns that limit routine clinical applications of genome-editing technologies. However, quality control measures are being developed to minimize off-target effects, DNA-damage toxicity and immunotoxicity, improve delivery methods and editing-efficiency, and thus provide more precise, more efficient and safer editing tools.

Keywords: Genome Editing, Meganucleases, Zinc-Finger Nucleases, TALENs, CRISPR.

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INTRODUCTION

The human genome consists of the entire sequence of deoxyribonucleic acid (DNA) nucleotides within the 46 chromosomes in the cell nucleus as well as a small chromosome in the mitochondria[1]. Every cell with a nucleus has a complete copy of the genome[2]. The term genome was created in 1920 by Hans Winkler, a professor of botany at the University of Hamburg, Germany. It was derived from two words, gene and chromosome. It includes the coding regions (≈2%), as well as the noncoding regions of DNA (≈98%), which include introns, non-coding RNA sequences, regulatory sequences, and repetitive DNA[1].

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Definition of Genome Editing

Involves making targeted and precise alterations (insertion, deletion or substitution) to a specific DNA sequence, regulating the activation or inactivation of gene expression and also rearranging chromosomes[3]. It can be carried out completely outside of the body in the laboratory (in vitro), within cells to be re-introduced into a living organism (ex vivo), or in the organism itself (in vivo)[4].

Basic Concepts

1. DNA (deoxyribonucleic acid)

DNA is a double helix molecule made up of nucleotides. A nucleotide consists of a five-carbon pentose sugar (deoxyribose), a phosphate group, and a nitrogenous base - pyrimidine (cytosine, thymine) or purine (adenine, guanine)[2]. The two long, thin strands of a DNA molecule are twisted around each other like a spiral staircase. The rails comprise a sugar-phosphate backbone while the steps comprise nitrogenous bases that occur in pairs, adenine with thymine and guanine with cytosine. A single DNA molecule is ≈ 3 billion base pairs long[1].

2. Gene

This is a section of DNA that encodes an RNA or protein product. It is the molecular unit of heredity. There are 20,000 to 25,000 protein-coding genes distributed between 23 distinct chromosomes of the haploid genome[2]. Two major components of a gene are the exons, which contain the protein-coding nucleotides, and introns, which comprise DNA sequences between the exons that do not code for proteins. All the exons within the genome are collectively referred to as the exome. The exome and introns comprise ≈ 2% and ≈25% of the genome respectively[1].

3. Nuclear Chromosome

A chromosome within the nucleus is a structure formed by a single linear DNA molecule (≈ 6 ft long) tightly wrapped around histone proteins. There are 23 pairs of nuclear chromosomes in every cell (44 autosomes and 2 sex chromosomes), which makes 46 chromosomes in total[5].

4. Mitochondrial Chromosome

This is a double stranded, circular DNA molecule of 16,569 base pairs. It contains 37 genes that encode 13 proteins, 22 transfer RNAs, and 2 ribosomal RNAs. The 13 proteins are subunits of the enzyme complexes of the oxidative phosphorylation system. There are several thousand copies of mitochondrial DNA (mtDNA) in a cell and they are normally inherited exclusively from the mother[6].

5. Karyotype

This is a photograph of a person's chromosomes arranged according to size. It is usually seen during mitosis at metaphase. The largest chromosome contains ≈ 8000 genes while the smallest chromosome contains ≈ 300 genes[5].

Historical Perspectives

The Sequencing and Mapping of The Human Genome

The process of sequencing identifies the order of the three billion chemical base pairs that make up human DNA while mapping identifies the location and determines the function of all the genes in the human genome[1,7,8].

Gene Sequencing

1. First generation (Sanger) Sequencing

Also known as 'chain-termination' or dideoxy technique, it was established by Frederick Sanger and fellow scientists in 1977[9]. DNA sequence is read one section at a time, usually short fragments of ≈ 300-1000 base pairs. After amplification via polymerase chain reaction (PCR), nucleotides are radioactively labeled and DNA fragments are separated with gel electrophoresis. The DNA bands may then be visualized by autoradiography or ultraviolet light, and the DNA sequence can be directly read off an X-ray film or gel image[10].

In the mid-1980s Leroy Hood improved on and automated the original Sanger method by introducing fluorescent dyes for labeling of oligonucleotides, capillary electrophoresis separation and fluorescent detectors, resulting in higher throughput (up to 384 DNA samples in a single batch)[9,10]. This enhanced the Human Genome Project (HGP) that was undertaken and ≈ 90% completed between 1990 and 2003[9].

2. Second or Next generation sequencing (NGS, 2004)

NGS involves various technologies that enabled rapid and high throughput sequencing of DNA and RNA. It resulted in massively parallel sequencing of millions of DNA fragments in multiple samples. With this method,

whole genome sequencing was done cheaper and more quickly, within days, rather than years that it took for the HGP[9].

3. Third-generation sequencing (TGS, 2011)

Real-time sequencing technology that allows direct sequencing of single DNA molecules. Unlike the Sanger and NGS methods, no polymerase chain reaction (PCR) amplification is required in the DNA library preparation[11].

4. Fourth-Generation Sequencing (FGS, 2015)

Combined single-molecule sequencing and nanopore technology enabling higher throughput, longer reads and lower costs[12]. The complete sequence of the human genome was eventually achieved in 2022[1].

Benefits of the Human Genome Project[7,8]

1. Provided insights into the genetic basis of diseases.
2. Facilitated the identification of mutations linked to different forms of cancer.
3. Revolutionized genetic and genomic research.
4. Led to the development of superior sequencing technologies.
5. Resulted in advances in the diagnosis and treatment of diseases.
6. Precision (personalized) medicine: the use of an individual's genetic profile to guide appropriate prevention, diagnosis and treatment of disease.
7. Pharmacogenomics: Identify patients that will benefit from a drug and those at risk for serious adverse effects based on their genetic profile.

Genome-editing Techniques

These techniques are mostly based on deliberately generating a double-strand break (DSB) at defined sites with a nuclease, which will trigger DNA repair mechanisms naturally present in cells via two endogenous DNA repair pathways – nonhomologous end-joining (NHEJ)-mediated and homology-directed (HDR) repair pathways[13]. In nonhomologous end joining repair, the broken ends are directly reattached, often resulting in the deletion or insertion of DNA sequences of varying lengths, which can disrupt the DNA sequence and inactivate the gene that was cut[14]. However, homology directed repair is more efficient and accurate because it makes use of a homologous template. If a homologous length of DNA is introduced into the cell as a donor template, or if specific alterations are included in this homologous template, it can introduce precise changes into the recipient genomic DNA[13,14].

Basic steps include:

1. Identify the specific DNA sequence in the human cell that needs to be targeted for editing[14].
2. Design customized genome-editing tools that can recognize and cut the target DNA sequence via protein engineering using recombinant DNA technology[14].
3. Testing and Optimization: tested in vitro (in cell-free systems) and in vivo (within cells) to ensure they bind to and cut the target DNA effectively[14].
4. Delivery into target cells with the use of viral vectors such as lentivirus and adeno-associated virus, which are commonly used, and non-viral vectors such as electroporation, microinjection and nanoparticles[3,14,15].
5. Double strand-DNA break and repair[3].

Genome-Editing Tools

Several genome-editing tools exist, such as meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems. The CRISPR-Cas (CRISPR-associated protein) system has proved most successful so far[3].

1. Meganucleases (MNs)

Meganucleases are endonucleases with high specificity and efficiency that recognize and cut large dsDNA sequences (12-40 base pairs) at specific sites. First identified in the early 1990s, they are naturally occurring restriction enzymes originally found in yeast and bacteria. The challenge encountered with their use is the difficulty in designing new meganucleases for different DNA sequences[14].

2. Zinc-finger Nucleases (ZFNs)

Zinc finger proteins (ZFPs) are naturally occurring proteins that recognize and bind to specific DNA sequences. Initially discovered in Xenopus frog in 1985, they are characterized by their use of zinc ions to stabilize their structural folds[13,15]. Since they do not have the ability to cut DNA, they were combined with the DNA-cleaving activity of the FokI restriction enzyme from bacteria to design ZFNs in the early 1990s. This improved the efficiency and specificity of genome editing[14]. However, this technique was difficult to use, time-consuming and expensive because a new ZFN must be engineered for each new target DNA sequence[14]

3. Transcription Activator-Like Effector Nucleases (TALENs)

TALEs are proteins found in Xanthomonas bacteria. FokI was fused to TALE proteins in 2011 to create TALENs[13,15]. They have similar efficiency and specificity as ZFNs but greater simplicity; less complex to design and assemble[14]. However, also expensive and complicated to use because each edit would require the construction of a new TALEN protein[14].

4. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

The CRISPR/Cas (CRISPR-associated) system was originally discovered in bacteria and archaea, where it functions as part of an adaptive immune system to protect bacteria from invading viruses. [14] CRISPR are bacterial DNA regions containing repetitive sequences interspersed with short, unique sequences called spacers derived from foreign (viral) DN[A3]. The main components of the CRISPR/Cas system are a single guide RNA (sgRNA), a DNA cutting enzyme and a healthy customized DNA sequence. The key feature that distinguishes it from previous techniques is that it uses RNA sequences instead of protein segments to recognize specific sequences in the DNA by complementary base pairing[14].

Cas proteins are the DNA cutting enzymes that work with CRISPR sequences to cleave specific nucleic acid sequences and they include Cas9 (designed in 2012), Cas12 and Cas13 (both designed in 2019). These 'genetic scissors' are much easier and faster to engineer, more efficient and more accurate in methodology and more precise in the genome sequence being targeted[3].

Current Applications of Human Genome-editing Technologies

The current applications of this technology include Research, Clinical Diagnosis and Clinical Therapy:

1. Research

Basic research involving genome editing of human cells (somatic and germline cells) and animal models such as mice, rats, zebrafish and pigs. This enhances the understanding of molecular processes involved in disease development and progression, and facilitates the identification of specific genes or DNA sequences that can be targeted for therapeutic intervention. Genome-editing research is currently ongoing in the following areas[3,13,16-18]

- Blood Diseases: examples include severe combined immunodeficiency syndrome and chronic granulomatous disease, amongst others.
- Cancer research
- Cardiovascular disease
- Neurodegenerative Diseases: Huntington’s disease, Alzheimer’s disease and Parkinson’s disease.
- Muscular Disorders: Spinal muscular atrophy, Duchenne muscular dystrophy.
- Metabolic Diseases: Tyrosinemia, alpha-1 antitrypsin deficiency, phenylketonuria, primary hyperoxaluria type 1 and type 1 diabetes.
- Respiratory diseases: Cystic fibrosis.
- Organ Transplantation: genome editing can modify donor organs to reduce rejection risks or improve compatibility with recipients.
- Fertility and prenatal treatment: genome editing can correct genetic errors in early embryos or germline cells.
- Regenerative medicine

2. Clinical Diagnosis

Diagnostic CRISPR systems have ultrahigh sensitivity and specificity. They have been demonstrated to have single-nucleotide specificity and versatility. They have been applied mostly in the diagnosis of infectious diseases, as well as cancer diagnosis and prognosis[13,17,18].

1. Infectious Diseases

The main focus of CRISPR-based diagnostics currently is the detection of nucleic acids of microorganisms[17,18]. Detection of the DNA or RNA of viruses, bacteria and parasites such as;

- RNA viruses: parvovirus B19, Flaviviridae (dengue, Zika and Japanese encephalitis virus), Ebola and Coronaviridae.
- DNA viruses: Herpesviridae (cytomegalovirus (CMV) and Epstein–Barr virus), Polyomaviridae (BK virus (BKV)) and Papillomaviridae (human papillomavirus).
- Bacteria: *Mycobacterium tuberculosis, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa* and *Salmonella* Enteritidis.
- Parasites: All *Plasmodium* species known to cause malaria in humans.
- Detection of antimicrobial resistance genes in clinical samples.

2. Cancer Diagnosis and Prognosis

Circulating (cell-free) tumor DNA and RNA, and non-nucleic acid tumor-derived proteins such as prostate specific antigen (PSA) can be detected by genome-editing techniques[17]. The single-nucleotide specificity of Cas enzymes enables the detection of point mutations and small deletions and aid in the early diagnosis of cancers such as high-risk HPV malignancies, prostate, breast, cervical, colon, lung, colorectal, and bladder cancers, medulloblastoma and melanoma. It also facilitates early detection of metastasis, cancer recurrence, and monitoring of treatment responses[17,18]. Many CRISPR assays are being validated by clinical trials, and assay validity is monitored and maintained after clinical implementation[17].

The main steps included in the protocol for nucleic acid detection include:

i. Sample Collection: Samples can be collected from saliva, nasopharyngeal secretions, blood or urine[18].
ii. Nucleic acid extraction (Sample Pre-Treatment): Nucleic acids are extracted using different methods such as heat and chemical lysis, or column-based extraction[18].
iii. Nucleic acid amplification: Preamplification via quantitative polymerase chain reaction (qPCR) or isothermal amplification strategy is required for nucleic acid concentrations below the picomolar (10-12 moles/L) range, for instance human immunodeficiency virus (HIV) or hepatitis C virus (HCV) load in patients undergoing antiviral therapy[17]. Nucleic acid concentrations above the picomolar range (relatively high DNA or RNA concentrations in a sample) do not require preamplification. For example, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the early phase of infection, human genomic DNA (gDNA), highly expressed human messenger RNAs and microRNAs (miRNAs)[17].
iv. Nucleic acid detection (Readout Options): Activation of the Cas enzyme upon target detection triggers collateral cleavage of fluorometric, colorimetric, biotin, or electrochemical reporters. The amplitude of the signal produced is proportional to the concentration of the target nucleic acid present in the sample[18].

Clinical Therapy

Many of the drugs approved for clinical therapy are undergoing clinical trials and those that their clinical trials have been successfully concluded are being used in authorized treatment centers to treat specified patients. Examples of conditions in these categories include:

1. Blood Diseases

The genome-editing drug, Casgevy (exagamglogene autotemcel), was the first CRISPR–Cas9 therapy approved in the UK (in November 2023), USA and Europe (both in December 2023), for the treatment of Sickle Cell Disease and Transfusion-Dependent Beta-Thalassemia, after successful clinical trials[19]. Casgevy functions by disrupting the BCL11A gene responsible for suppressing fetal hemoglobin (HbF) production, resulting in high levels of HbF in red blood cells. This produces a curative, one-time treatment for patients[13,15,20]. Another genome-editing drug, Lyfgenia (lovotibeglogene autotemcel), which also received regulatory approval for clinical use in December 2023, genetically modifies the HBB (β-globin) gene in patient's blood stem cells to produce a form of hemoglobin A (HbA^{T87Q}) that is resistant to sickling[19,20]. Genome-editing therapy has also been developed for hemophilia[13,16].

2. Cancer prevention and Therapy

Genome-editing technologies target genetic and epigenetic mutations in oncogenes and tumor suppressor genes. They help to overcome cancer therapeutic resistance and sensitize tumors to existing therapies[13]. There are many ongoing clinical trials using CRISPR-based therapies for leukemia, lymphoma, brain cancer, renal cell carcinoma, colorectal cancer, hepatocellular carcinoma, urinary bladder cancer, lung and esophageal cancers[13,16].

3. Infectious Diseases

Genome-editing knocks out multidrug-resistance (MDR) genes and vaccine-escape mutations and has been used in the treatment of COVID-19, Human Immunodeficiency Virus (HIV), Human Papilloma Virus (HPV), HBV, TB, Zika, and dengue[13,16]. The first genome-edited babies (twin girls) were born in China in 2018. A Chinese scientist used CRISPR/Cas9 to edit the CCR5 gene in the embryos in order to produce resistance to HIV[13,21].

4. Other Conditions[13,16]

- Inherited eye disorders: Leber congenital amaurosis (LCA, inherited retinal degeneration), Retinitis pigmentosa (RP).
- Auditory Disorders: Congenital hearing loss
- Metabolic Diseases: Hypercholesterolemia, Transthyretin amyloidosis.
- Immune Response Disorders: Hereditary angioedema (HAE)

5. Vaccine Production

Key ways in which CRISPR/Cas technology can contribute to vaccine production include:

i. Create animal and cell models for vaccine development research and vaccine testing[22].
ii. More efficient and targeted vaccine design: generate recombinant vaccine vectors and edit the genes of B-cells to produce specific antibodies[22,23].
iii. Large scale vaccine production at lower cost[23].
iv. Improved vaccine safety and efficacy[22,23].

Technical Challenges

These factors limit clinical applications of genome-editing therapies by affecting editing-efficiency[3,13].

1. The target cell type and cellular uptake.
2. Choice of genome-editing tools.
3. Technologies for delivering editing components.
4. Product degradation.
5. Immunogenic effects of genome-editing proteins or delivery reagents.

Ethical Concerns

1. Safety: The potential risks associated with genome-editing therapy include[3,13,15,24]:
    - Off-target effects (edits in the wrong place)
    - Unintended on-target edits (unexpected gene expression)
    - Mosaicism (when some cells carry the edit but others do not)
    - Cellular toxicity and genome instability: DNA-damage toxicity mediated by the tumor suppressor gene p53.
    - Immunogenicity (a negative immune response)
2. Genome-editing research involving embryos: moral and religious objections[3,13,15]
3. Clinical reproductive purposes: Germline cell and embryo genome editing can be passed to future generations and are currently illegal in many countries[3,13,15]
4. Use of genome editing for non-therapeutic and enhancement purposes: athletic ability, designer babies[14].
5. Access and equity: Current genome-editing therapy is very expensive and may be accessible only to those that can afford it[14].

Quality Control (QC) in Genome-editing Technology

Quality Control in genome-editing techniques can be established by the following measures[25-28]:

1. Validation of targeting efficiency: Sequencing (Sanger, NGS), genotyping (PCR).
2. Off-Target analysis: Bioinformatics tools, deep sequencing (the entire genome or targeted regions).
3. Functional testing: Phenotypic Analysis, Expression Studies (Quantitative PCR, Western blotting).
4. Cellular and Molecular Profiling: Flow Cytometry, Transcriptomic and Proteomic Analysis.
5. Safety Testing: In Vivo Studies (Animal models or other biological systems), Biological Safety Testing.

Strategies for reducing off-target effects, genomic rearrangements, DNA-damage toxicity and immunotoxicity include[24,26-28]:

1. Single-guide RNA (sgRNA) modifications
2. Cas9 protein optimizations
3. Cas9 system modifications
4. Improved Cas-nuclease variants
5. Use of anti-CRISPR proteins
6. Improvement of delivery methods
7. DSB-independent gene editing: use of base editors, prime editors or epigenetic editors.

CONCLUSION

Human genome-editing is a cutting-edge technology that has made dramatic advances in research and therapy, and it vastly improves on existing diagnostic methods. Work is still ongoing to overcome technical barriers, safety and bioethical concerns and move its use beyond clinical trials to routine clinics within the shortest possible time.

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