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Is epigenome editing non-inheritable? Implications for ethics and the regulation of human applications

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Epigenome editing offers ethical advantages with non-inheritable gene expression control. However, concerns arise regarding potential transgenerational effects in humans. Ethical and regulatory evaluation is crucial, considering recent advancements and enhanced understanding of transgenerational epigenetics in both mammals and humans.

INTRODUCTION

Epigenome editing is a technology that regulates gene function by artificially controlling epigenetic states at specific locations on the genome. It has been recognized as a promising therapeutic approach for genetic disorders and chronic diseases. A recent study suggested that epigenome editing, which does not alter the genome sequence and has reversible intervention effects, poses fewer ethical issues than permanent or irreversible genome editing (Zeps et al., 2021). According to Zeps et al., the impact of epigenome editing on the germ cell lineage is also minimal.

However, another study suggested that epigenetic inheritance across generations is possible in mammals (Takahashi et al., 2023). Technologies have also been developed to maintain artificial gene expression control as epigenetic memory with transient epigenetic interventions. Under these conditions, the effects of epigenome editing can likely be passed on to the next generation in some form. If this is true, it may be premature to claim that any type of epigenome editing poses fewer ethical issues than genome editing.

We agree that genome editing and epigenome editing require similar regulation for somatic interventions; however, we call for a more comprehensive discussion on the ethics and regulation of clinical applications of epigenome editing in humans.

EPIGENOME AND EPIGENETIC INHERITANCE

The genome contains genes, regulatory elements, and structural regions. This sequence of information needs to be precisely maintained for the development, homeostasis, and reproduction of the organism. It is also essential that such sequence information functions under strict spatiotemporal control.

The overall functional regulatory information of the genome (not involving genetic changes) is known as the epigenome. Epigenetics, the regulatory mechanism of the epigenome, is responsible for the quantitative control of gene expression. The molecular factors involved in the epigenetic processes include DNA methylation, histone modifications, non-coding RNAs, chromatin three-dimensional (3D) conformation, and transcription factor binding (Fitz-James and Cavalli, 2022). These factors are regulated by corresponding enzymes and/or other molecular entities in a sequence-dependent manner in the genome, and multiple factors work together, resulting in diverse and precise transcriptome regulation. Disorganization of these mechanisms leads to genetic disorders and cancers.

Epigenetic changes in response to environmental stimuli can also play a role in regulating genome function. Transgenerational epigenetic inheritance (TEI) has been observed in several organisms, including yeast, plants, and nematodes. Environmentally induced epigenetic changes are transmitted from one generation to the next (Fitz-James and Cavalli, 2022; Lacal and Ventura, 2018). However, whether TEI occurs in mammals remains unclear. Major epigenetic features, such as DNA methylation and histone modifications, undergo reprogramming during mammalian germ cell development and early embryogenesis (except for certain loci, e.g., imprinting regions). Epigenetic information acquired by parents through environmental stimuli is assumed to be erased during this process.

However, a recent experimental study suggested that artificially introduced DNA methylation states at the embryonic stage are reconstructed after epigenomic reprogramming in mice (Takahashi et al., 2023). These states (accompanied by phenotypic effects) can be maintained in multiple generations of the offspring. Nonetheless, how, where, and to what extent

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Table 1. Epigenome editing techniques and their characteristics

Category	Description	Advantages	Disadvantages	Examples to target diseases	
				Tools	Diseases
"On-site-only" epigenome editing	• temporal editing effect only occurs when editors are expressed in cells by directing the enzymatic activity of epigenetic effectors	• relatively simple editing tools • relatively easy to halt the effects of interventions, including off-target effects	• requires durable delivery systems such as AAV vectors • higher risks for immunogenicity • safety concerns related to possible genotoxicity due to unintended vector integration (at low frequency)	• CRISPRa • CRISPRi • dCas9-Tet1 • dCpf1-CTCF • dCas9-DNMTs • counterparts with TALEs and ZFPs	• congenital muscular dystrophy (LAMA2 gene) • fragile X syndrome (FMR1 gene) • Rett syndrome (MECP2 gene) • facioscapulohumeral muscular dystrophy (DUX4 gene) • various cancers
"Memory-forming" epigenome editing	• persistent editing effect, which remains after editors are removed because of the synergistic effect of epigenetic effectors to induce epigenetic memory	• continuous intervention is not required, allowing non-viral transient delivery • lower risks for immunogenicity	• more complex editing tools using multiple effectors • active reintervention is required to reverse the effect, including off-target effects • potential "inheritability" issues in the germline/ embryonic interventions	• Hit-and-run silencing • CRISPRoff, (CRISPRon)	• hyperlipidemia (PCSK9 gene) • chemotherapy-induced peripheral neuropathy (SCN9A gene)

CRISPRa, CRISPR activation; CRISPRi, CRISPR interference; DNMT, DNA methyltransferase; TALE, transcription activator-like effector; ZFP, zinc-finger protein.

this epigenetic memory is transmitted across generations remains unclear. A comprehensive picture of mammalian and human epigenomic inheritance is currently unavailable, warranting rigorous scientific investigation. Given this uncertainty, the possibility that artificial epigenetic modifications, especially at germline and embryonic stages, affect future generations should not be underestimated.

Epigenome editing

Epigenome editing aims to adjust genome function (mainly gene expression) without altering the genomic sequence. Epigenome editing has shown rapid advances, alongside the development of genome editing. It has become easier to achieve sequence-specific local epigenetic control. This is performed by using programmable DNA-binding proteins such as CRISPR-dCas9 (a catalytically inactivated Cas9), in which the DNA-binding function is retained but the cutting function of genome editing tools is removed. When fused with epigenetic enzymatic domains (also known as effector domains), these DNA-binding proteins can exert local enzymatic effects and epigenetic control (Chang and Qi, 2023). An increasing number of studies are using such epigenome editing techniques to modify disease-specific epigenetic features in non-disease states or adjust gene expression levels to treat cancers and genetic disorders (Table 1).

"On-site-only" epigenome editing

Recently, several epigenome editing applications have been reported. For example, dCas9-Tet1 downregulates DNA methylation; it can reactivate FMR1, a gene that is silenced in fragile X syndrome (FXS), and improve neural functioning in FXS patient cell models (Liu et al., 2018). dCpf1-CTCF is a catalytically dead Cpf1 fused with a CCCTC-binding factor that regulates the local chromatin structure. In Rett syndrome, dCpf1-CTCF can enhance MECP2 reactivation via dCas9-Tet1 (Qian et al., 2023).

CRISPR activation (CRISPRa) is a transcriptional activation system. Not only disease-causing genes but intact disease-modifier genes can be activated by CRISPRa, exemplified by the phenotypic improvement in a mouse model of congenital muscular dystrophy (Kemaladewi et al., 2019). This case should be considered a therapeutic intervention rather than an enhancement. CRISPR interference (CRISPRi) is a transcriptional repression system that has been applied to suppress the causative genes of muscular dystrophy and cancers, and a clinical trial based on this principle has been planned (Himeda et al., 2021).

These effects are transient only when the tools are inside the nuclei. As such, durable delivery agents that remain in the body for a long period (e.g., adeno-associated viral or lentiviral vectors) are required. However, this raises safety concerns, such as genotoxicity attributable to unintended

integration and liver dysfunction. Frequent administration using non-viral methods might help overcome the limitation of transient effects. Nonetheless, this could increase the burden on patients. In such cases, stopping the intervention or removing the tools could cause the effects (including off-target effects) to disappear. Therefore, this type of epigenome editing intervention and oral medicine are thought to share a similar level of reversibility in effects.

"Memory-forming" epigenome editing

Hit-and-run silencing and CRISPRoff induce epigenetic memory with a local heterochromatin signature, including DNA hypermethylation and H3K9me3, by transient intervention, thereby maintaining persistent gene silencing even after the editing tool is removed, at least during cell division and differentiation (Amabile et al., 2016; Nuñez et al., 2021). The application of this approach in disease treatment is promising because continuous intervention is not required. In such cases, the delivery method may also apply a transient non-viral form. Efforts to develop a method to achieve stable gene activation with a single administration are also under way (Chang and Qi, 2023). Although these induced persistent effects can be epigenetically reversed, reintervention in the opposite direction by chemically distinct enzymatic activity is required to halt them. For reversibility, this intervention is not comparable with the "on-site-only" epigenome editing described above; it does not passively return to the pre-intervention state. Rather, this type of epigenome editing may display irreversibility observed in genome editing. This clearly falls outside the scope of features of epigenome editing envisioned by Zeps et al.

TEI has been demonstrated in mammals. Therefore, we cannot rule out the possibility that the effects of epigenome editing are transgenerationally inheritable in the germline. However, further research is required to confirm this. Germline applications of epigenome editing have not been reported so far. Nonetheless, the theoretical possibilities for ways in germ cell lineages include (1) direct intervention in germ cells, (2) intervention in prenatal fetuses with subsequent effects on their germ cell lineages, (3) postnatal infants with subsequent effects on their germ cell lineages, and (4) currently unlikely but inadvertent contamination of epigenome editing tools in germ cells through systemic somatic intervention.

The traditional debate on the ethics and regulation of human genome editing has focused on editing the human embryo for clinical purposes because of concerns about inheritable effects for future generations. Similar to heritable genome editing, it is impossible to avoid ethical issues surrounding the effects of epigenome editing being inherited by the next generation if there is a possibility that these effects will act on germline cells.

Off-target effects

Similar to genome editing, epigenome editing exerts potential selective off-target effects in the form of unintended effects on genome-wide regions. Moreover, unlike Cas9 nuclease activity, the effector domain is exposed in the nuclei, even when it is not targeted. This raises concerns regarding random (non-selective) off-target effects. Ideally, the long-term off-target effects must be investigated in cases in which constitutive introduction is required or inheritable methods (e.g., hit-and-run editing) are used.

Thus, the phenotypic influence of selective and non-selective off-target effects may spread. These effects may also have unforeseen implications for the human population if epigenome editing becomes widespread in the medical field. Moreover, the outcome of epigenetic effects can be measured as a quantitative analog change of associated epigenetic factors rather than as a digital outcome of a genetic change of A/T/G/C. This makes it difficult to determine clear criteria to judge the safety.

Numerous regulatory elements (e.g., enhancers on the genome) exist, the epigenetic alterations of which bring about subtle changes in an individual's biological traits (Claringbould and Zaugg, 2021). The off-target effects of epigenome editing may influence phenotypes through such regulatory elements. Furthermore, the influences of parental environmental stimuli can be transmitted across generations in humans (as exemplified by the Dutch famine, in which malnutrition in mothers during pregnancy affected the metabolism of their descendants for generations). However, it is not yet clear whether such transmission occurs only through germ cells. Components outside the reproductive cell lineage (e.g., the placenta) may also be involved in routes of transgenerational epigenetic transmission (Lacal and Ventura, 2018; Sailasree et al., 2017). Considering the roles of endogenous retroviruses (ERVs) in the placenta and the repurposing of the ERV silencing machinery for "memory-forming" epigenetic silencing, we might need to assume infertility or developmental disorders through dysfunction of the placenta as potential side effects if pregnancy is desired in the future, after systemic interventions of such epigenome editing.

Given that environmentally induced epigenetic changes can be transmitted across generations, unpredictable changes resulting from epigenome editing may affect the offspring. As with genome editing, it may then be necessary to consider selective off-target avoidance depending on individual variants. This could alleviate concerns regarding the differences between reference genomes and individual genomes (Cancellieri et al., 2023). Thus, we need to consider a wide range of factors for constructive ethical and regulatory discussions to accurately estimate the

relevant effects, along with the off-target effects of inheritable epigenome editing as a serious ethical issue. Such considerations are similar to those traditionally identified for heritable genome editing in the reproductive cell lineage.

CLOSING REMARKS

There is a diversity of epigenome editing tools and an increasing number of ways in which they may be applied in humans. The notion of TEI is becoming increasingly established. Therefore, it seems misguided to discuss the ethics and regulation of epigenome editing simply as a non-inheritable technology. The inheritability concerns and potential risks associated with epigenome editing's inheritability necessitates rigorous experimental investigation.

We contend that each application of epigenome editing should be accompanied by careful consideration of the epigenetic effects used, the persistence of such effects, and associated delivery methods. The effectiveness with which we can assess the safety of and effects on future generations is limited. Nonetheless, the following criteria appear to be realistic for assessing the validity of human applications for disease treatment: (1) selectivity of the relevant intervention on target tissues or cells, (2) possibility of direct effects on the germline, and (3) severity of disease symptoms.

These criteria also apply to the extension of existing therapies (defined by Zeps et al.). The epigenome plays a pivotal role in myriad biological processes, encompassing physical traits unrelated to the disease, physical and intellectual abilities, environmental adaptation, and aging. Although the potential for epigenome editing to enhance or alter these traits exists, an exploration of the ethical ramifications of such actions is beyond the purview of this article. Inheritable epigenomic

interventions may be appealing from the perspective of preventing disease transmission. However, they pose enduring ethical and legal dilemmas surrounding the impact on future generations, similar to heritable genome editing (Huerne et al., 2022). Therefore, we advocate the establishment of stringent regulations analogous to those governing heritable genome editing and gene therapy, especially for future inheritable epigenome editing and embryonic interventions.

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AUTHOR CONTRIBUTIONS

All authors collaboratively conceptualized this project. M.S.-H. drafted the manuscript with supervision by K.A. and T.S. The authors jointly participated in the editorial process.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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