ISSN 2816-8089

REVIEW ARTICLE

A review on Epigenome Editing using CRISPR-based Tools to Rejuvenate Skin Tissues

Ali Saber Sichani[1], Maryam Baneshi[2], Maryam Ranjbar[3], Yasaman Naeimzadeh[4], Jafar Fallahi[4*]

[1]*Department of Biology, Texas A&M University, College Station, TX, 77843, USA.*
[2]*Department of Medical Genetics, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.*
[3]*Department of Medical Biotechnology, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran.*
[4]*Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies. Shiraz University of Medical Sciences, Shiraz, Iran.*

Abstract

Genomic activity is controlled by a sophisticated series of cell functions known as the epigenome. The creation of tools capable of directly altering various processes is required to unravel this intricacy. Additionally, by employing tailored DNA-binding platforms connected with effector domains to serve as targeted transcription factors or epigenetic modifiers, it is possible to control the chemical modifiers that regulate the genome's activity. Neoplastic disorders have received the most attention in the study of epigenetics, though the epigenome's significance in a variety of disease processes is now well acknowledged. Researchers are inspired to investigate novel approaches to revert these pathogenic alterations to their normal patterns by considering the fact that the epigenome profile of individuals with aging skin cells or other skin disorders, including atopic dermatitis, differs from that of healthy individuals. Here in this review, we discuss the use of CRISPR/dCas9 as a cutting-edge and flexible tool for fundamental studies on chromatin structure, transcription regulation, and epigenetic landscapes, as well as the potential of this method in these fields. Furthermore, we review on common and recently invented methods to make epigenetic alterations possible in daughter cells after any mitotic differentiations. In the very near future, CRISPR-based epigenomic editing will become a potent tool for comprehending and regulating biological functions.

Key Words: *Epigenetics; CRISPR/Cas9; Epigenetic editing; Rejuvenation; Keratinocyte; Skin*

***Corresponding Author**: Jafar Fallahi, Shiraz University of Medical Sciences, Shiraz, Iran; Email: jafarf80@gmail.com*

***Received Date**: April 27, 2023, **Accepted Date**: May 16, 2023, **Published Date**: June 09, 2023*

***Citation:** Sichani AS, Baneshi M, Ranjbar M, et al. A review on Epigenome Editing using CRISPR-based Tools to Rejuvenate Skin Tissues. Int J Bioinfor Intell Comput. 2023;2(2):142-157.*

1. Introduction

Multicellular organisms have almost identical genomic sequences in all their cells, yet various cells can continue their respective roles throughout the organism's lifetime. An attempt to assess the source of this diversity has instituted the field of epigenetics, which encompasses a wide variety of factors beyond the DNA sequence that regulate genomic activity [1]. Chromatin accessibility [2], biochemical modification of DNA and histones, RNA and protein factors modulation, and DNA element interaction are examples of epigenetic factors [3-5]. These factors in combination create an 'epigenetic state' of the cell. Linkages between epigenetic state, gene expression, and cell fate suggest correlations between epigenetics and cellular phenotype [6-8]. Targeting epigenetic modifiers on specific genes might be a safer option than gene editing. Tethering these effectors to sequence-specific DNA-binding domains (DBDs) allow for targeted epigenetic changes. This localizes the epigenetic change near the DBD-bound site, establishing links between the presence of effectors, induced epigenetic alteration and gene regulation. Using of DBDs has laid the basis for targeted epigenetic engineering. However, DBD-based approaches have some limitations, such as difficulty to target a wide range of genomic loci, because targeting a different DNA sequence requires a corresponding distinct protein [6,9-12]. CRISPR/Cas systems have evolved into powerful tools for targeted genetic changes [13,14]. Although CRISPR-based methods are being invented in by pioneer scientists [15], the common CRISPR/Cas9 system is also being improved consistently that could lead to better efficiencies, targeting practically any desired sequence [16]. The reprogramming of CRISPR systems has enabled the creation of a wide range of epigenome engineering methods. Despite DBDs, CRISPR-associated (Cas) proteins achieve genomic specificity via their small guide RNAs (gRNAs). Most Cas proteins carry out nucleases that cleave the targeted DNA sequence. In 2013, Lei S. Qi et al. repurposed CRISPR as a platform to make it functional for epigenetic editing. Mutations in genes that encode Cas proteins can result in nuclease-deactivated or dead Cas (dCas) proteins, which still retain their ability to bind any desired part of the genome using a guide RNA without introducing a break [17]. Along with CRISPR/Cas9-based techniques, there has been a lot of progress with dCas9 tools that allow the recruitment of specific protein functions to the target genomic regions with a lot of flexibility [18,19]. A single dCas can then be reprogrammed to target a new locus simply by changing its gRNA sequence (Figure 1), resulting in a rapidly adaptable platform for the localized targeting of practically any genomic sequence [6].

2. Epigenome Editing

The precision with which the epigenome can now be manipulated via CRISPR/Cas systems has significantly accelerated mechanistic epigenetics and created new tools for more accurate models of human diseases. The role of the epigenome in disease can be explained while also providing treatment approaches for restoring healthy phenotypes by modeling epigenetic aberrations identified in disorders using epigenome editing [20,21]. Epigenome editing intends to write or erase specific epigenome marks at specific gene loci to alter gene expression. This tool has allowed researchers to fuse dCas9 with epigenetic writers and erasers [22-24]. They were able to keep the marks from one generation of cells to the next by merging several epigenetic effectors [25,26]. Programmable locus-specific DNA-targeting methods are crucial in achieving these goals, which enable the genome locus-specific recruitment of specific editing of epigenome modifiers [10].

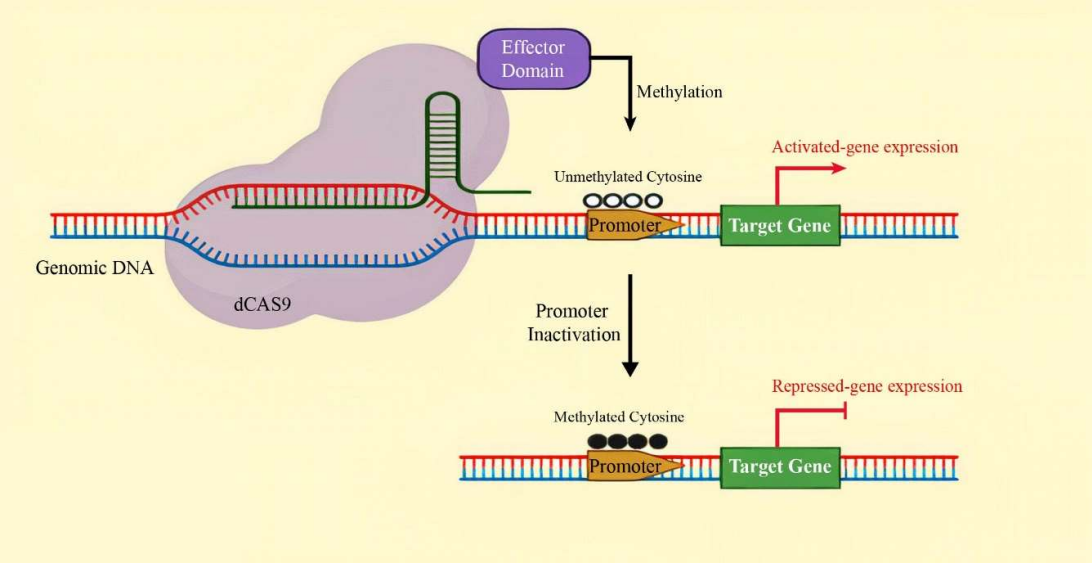

Figure 1: *The complex of a dCas9, sgRNA, and a related effector domain can attach to the targeted genomic sequence and then methylate the cytosines on a promoter to silence the gene without altering its DNA.*

In the field of medicine, epigenetic abnormalities have been found to be causative factors in cancer, genetic disorders, and pediatric syndromes as well as contributing factors in autoimmune diseases, aging, and skin diseases [27-29]. Research has shown that these epigenetic regulation mechanisms play an important role in controlling cellular functions, including keratinocytes, and the pathogenesis of skin diseases [30]. Skin heals through a dynamic and coordinated process of cell proliferation, migration, and differentiation [31]. Much research show that epigenetic pathways together with several transcription factors and signaling pathways play a key role in regulating various aspects of epidermal growth and keratinocyte differentiation which have been seen during skin repair [32,33]. Currently, virtually all of the research in this field are focused on understanding the interactions between transcription factors and epigenetic regulatory machinery, in controlling epidermal growth, regeneration, and stem cell activity [34,35]. These efforts are important to answer the challenge of how epigenetic reprogramming enables the re-regulation of repair genes. In the present review, in the first part, we aim to summarize the available evidence and studies on the regulation of epigenetic mechanisms of chromatin, and the second part focuses on our current understanding of different types of epigenetic changes in both keratinocyte differentiations from their progenitor cells and the epigenetic changes in skin diseases. To gain better knowledge in this field, the five exclusive groups of epigenome engineering technologies using CRISPR/dCas9 include long-range chromosomal interactions such as chromatin remodeling, transcriptional control, histone modification, and DNA modification have comprehensively been explained [36,37].

2.1. Transcriptional activator

The first attempt at epigenome editing was transcriptional regulation. According to their actions, transcription factors fused to dCas9 can be classified as transcriptional activators or transcriptional repressors. The CRISPR/dCas9 methods using these effectors are known as CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi), respectively [38,39]. Transcriptional activators, including p65, HSF1, Rta, and VP16, VP16 multimers (including

VP48, VP64, and VP160), boost the recruitment of chromatin modifiers, which leads to chromatin decondensation, the accumulation of histone marks like acetylation of histone H3 at lysine 27 (H3K27ac) and trimethylation of histone H3 at lysine 4 (H3K4me3), RNA polymerase II (Pol II) binding and subsequent mRNA transcription. The dCas9 can promote robust gene activation by employing modular methods to engage various activation domains. Among VP16 multimers, VP64 is the most commonly used and is a more rousted activator than a single VP16. HSF1, p65, Rta, and VP64 are commonly employed as a combination to achieve better editing effects. For instance, VP64p65-Rta (VPR) is preferable to VP64 in both up-regulation consequences and editing specificity [40].

2.2. Transcriptional repressor

Krüppel-associated box (KRAB), mSin3 interaction domain (SID), and FOG1 are transcriptional repressors that work by recruiting suppressive transcriptional factors or histone modification enzymes. For instance, KRAB, the most often employed repression domain in epigenome editing, attracts HP1 and KAP1/TRIM28 proteins, preventing Pol II from being positioned. These elements also stimulate the recruitment of histone methyltransferases, which cause local chromatin compaction by increasing the levels of trimethylation of histone H3 at lysine 9 (H3K9me3). But at the other hand, KRAB is less specific and can sometimes have a broad effect on the cellular state. LSD1 may be better at editing specificity than KRAB. In some studies, KRAB is utilized in cooperation with other EpiEffectors (like DNMT) and results in durable and highly precise epigenetic silencing of endogenous genes [41-43]. The SID recruits histone deacetylases via interaction with PAH2, the transcriptional repressor domain, and is not yet widely used [42].

2.3. DNA methylation and demethylation

In mammalian cells, adding a methyl group to the 5th carbon of cytosine (DNA methylation) has a proven effect on the regulation of gene expression. CpG islands (CGIs) are abundant in mammalian promoter regions, with more than 50% frequency of CpG nucleotides. Transcriptional inhibition has been connected to the methylation of CGI loci near a gene's transcription start point [44].

The de novo methyltransferases DNMT3A and DNMT3B, in addition to maintenance methyltransferase DNMT1, are responsible for inducing and maintaining cytosine methylation. Although DNMT3L lacks methyltransferase activity, it acts as a cofactor for DNA methylation [45]. DNMT3A and DNMT3B have been used as effectors by CRISPR/dCas9 in epigenome editing. DNMT3A and DNMT3B, the de novo methyltransferases, in addition to maintenance methyltransferase DNMT1, are responsible for inducing and maintaining cytosine methylation. Despite the lack of methyltransferase activity of DNMT3L, it acts as a cofactor for DNA methylation. DNMT3A and DNMT3B have been used as effectors by CRISPR/dCas9 in epigenome editing.

Moreover, prokaryotic DNA methyltransferase MQ1 has also been used in epigenome engineering. MQ1 includes 386 amino acids and is a de novo DNA methyltransferase with high efficiency [46]. Additionally, DNA demethylation enzymes, like the ten-eleven translocation 1 (TET1), have been used in epigenome editing. For instance, demethylation of DNA in the BRCA1 promoter by dCas9–TET1 both rescued expression and inhibited cell

proliferation in a cancer cell line that proves epigenome editing can fix hypermethylation of tumor suppressor gene promoters, which is one of cancer's hallmarks [47,48].

2.4. Histone modification enzyme

As we discussed earlier, epigenetics is controlled by histone modifications that condense chromatin fibers around themselves to repress a gene (Figure 2). Histones can carry a variety of markers that change the structure of chromatin, the DNA-histone complex that loops and whorls through the nucleus in cells. Genes that reside in densely packed chromatin tend to stay silent. Genes that exhibit more open stretches tend to be transcribed [26]. Various histone proteins (like H2A, H2B, H3, and H4) organize genomic DNA on the nucleosomal scale. Post-translational modifications occur in specific histone residues (for example, lysine (K) 9 or K27 of H3). A large number of these changes have been linked to distinct gene expression profiles [25,49].

Effector proteins modify specific histone residues by adding or removing covalent groups, inducing phosphorylation, acetylation, methylation (mono-, di-, and trimethylation), and ubiquitination. The exact transcriptional consequence related to each histone modification is defined by the cellular environment.

Methylation (DOT1L, PRDM9, G9A, SUV39H1, and Ezh2), demethylation (LSD1), acetylation (p300), and deacetylation (HDAC) are all frequent in CRISPR/Cas9 epigenome editing. CCCTC-binding factor (CTCF) was altered successfully by using a mutant version of dCas9-MQ1 to target DNA methylation. Specific histone modifications, such as H3K4me3 and H3K27me3, are related to gene activation and repression, respectively [50].

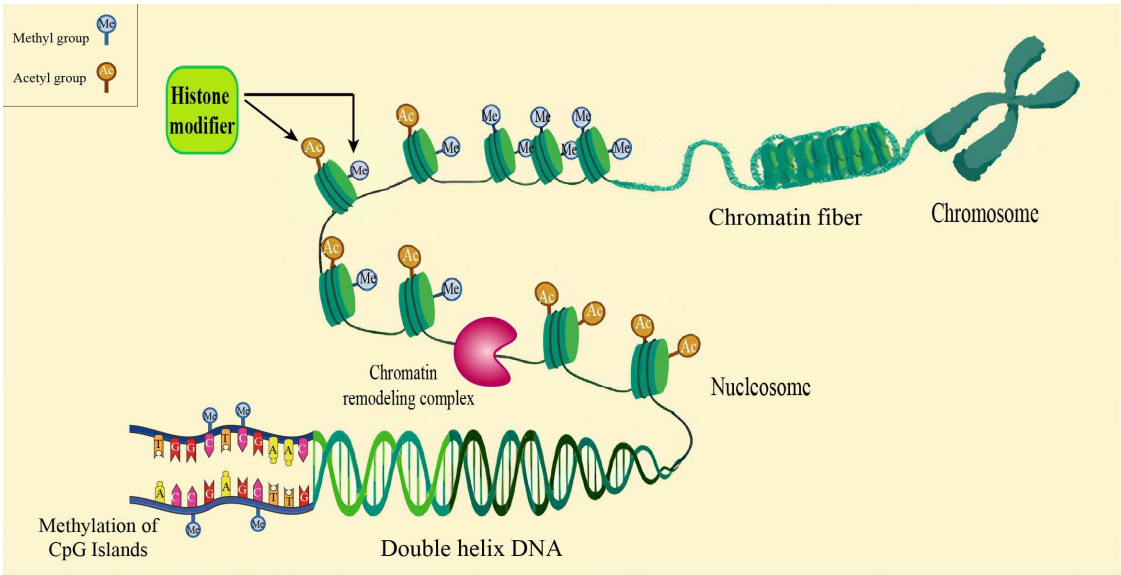

Figure 2: *Naturally, histone modifiers inside the nucleus have the role to proceed with methylation, demethylation, acetylation, etc. on the CpG islands. With the help of histones and chromatin remodeling complexes, a chromatin fiber can be condensed or decondensed at specific locations to make a gene be repressed or transcribed, respectively.*

2.5. Epigenetic changes in mtDNA

The mitochondria are signaling organelles that transmit information about their health to the nucleus. This information activates transcriptional regulators that control to homeostatic stress, which is crucial for organismal health and aging. Changed levels of mitochondrial metabolites or stress signals generate numerous epigenetic alterations that aid in maintaining cell homeostasis and have an impact on cellular aging [51], and methylation changes are reversed by the restoration of mtDNA in the lab [52]. Since mtDNA does not have any histones, long and short noncoding RNAs, and post-translational changes of nucleoid proteins regulate the epigenetic status of mtDNA. Alterations in metabolism, circadian rhythm disruptions, and cancer can all be caused by mitochondrial malfunction, which manifests as changed gene expression and ATP generation as a result of epigenetic modifications [52].

2.6. Chromatin editing

One significant aspect of epigenetic engineering is manipulating the expression of genes through chromatin editing [53]. Efficient chromatin engineering will entail the combinational use of complementing effectors. Besides DNA methylation, searchers have illustrated the concept of complementary domains providing better activity for developing CRISPRi/a technologies, including increased activity by integrating chromatin editors with CRISPRi/a [54,55].

Eventually, achieving the most efficient engineering may require simultaneously targeting multiple loci, each with its specific epigenetic modifications, which are most easily accomplished using gRNA-specific domain-recruitment technologies [56,57]. A vast tapestry of chromatin markers is almost underexplored. For example, H2A ubiquitination, which is relevant to gene silencing, has not been scrutinized from a chromatin-editing perspective [58]. There are other marks like acetylation and methylation at other histone sites and also additional chemical modifications, including crotonylation [59], phosphorylation [60], propionylation [61], and DNA adenine N6 methylation (m6A) [62]. These modifications interact with well-studied markers, revealing natural candidates for combinatorial chromatin editing.

2.7. Enhancers

Researchers also use targeted epigenetic editing to comprehend the role of enhancers, which are small regions of the genome that activate genes from afar. An enhancer could be located near a gene or on a different chromosome entirely. As a result, it is exceedingly challenging to tell which genes any enhancer is regulating. Up to this point, it's been pretty hard to study enhancers and figure out what they do because there is no tool to go in and disrupt them. But now, epigenome editing tools have made this possible. The same epigenetic markers that affect gene expression also suppress or activate enhancers [26]. In 2015, Gersbach et al. revealed that individual enhancers could be turned on by hitching dCas9 to enzymes that add acetyl groups on histones and then directing these fusion proteins to particular enhancers. The human body could have up to two million different enhancers, and trying to manipulate them one by one would take an eternity [63].

3. Epigenetic and Skin Disorders

The epidermis forms the surface layer of the skin and is made of specialized epithelial cells called keratinocytes, which are organized into several layers [64]. These keratinocytes undergo complex differentiation processes, morphological and metabolic changes, along with extensive changes in gene expression, and acquire distinctive features that are necessary for the formation of the outer barrier of the body (epidermis). This process, known as epidermal differentiation, is very important to maintain the epidermis under physiological conditions as well as stress or in various skin pathologies [65,66]. Epidermal differentiation relies on a highly coordinated program of gene expression [67-69]. In addition to these regulators, epigenetic regulation through DNA methylation, histone modifications, and miRNA activity also play an important role in the process of keratinocyte differentiation and epidermal layering during homeostasis and after wound injuries [70,71].

Epigenetic mechanisms, also modulate various stages of gene expression by changing chromatin accessibility and mRNA stability [72,73]. Disturbance in these epidermal differentiation processes causes several skin diseases. Extensive studies have been conducted on transcriptional regulation related to epidermal differentiation and several signaling pathways and transcription factors including p63 and AP1 have been identified as key regulators of epidermal keratinocyte survival and differentiation and important drivers of cancer development [74,75].

For example, studies have shown that deletion of DNMT1 leads to premature keratinocyte differentiation, thickened epidermis, and alopecia, along with reduced hair follicle stem cell activity [76]. In addition, the deletion of SETD8 (a histone methyltransferase), JMJD3 (histone demethylase), and HDAC1/2 causes disruption of epidermal layering. Meanwhile, increasing the expression of JMJD3 leads to the acceleration of the differentiation process [77,78]. These changes in the regulation of the epigenetic system have led to various cancers and skin syndromes such as CYLD cutaneous syndrome [79]. Research shows that the mentioned skin disease is caused by frequent mutations in the epigenetic regulators DNAM3A and BCOR along with biallelic mutations in the CYLD as a tumor suppressor gene [80,81].

In recent years, the potential role of epigenetic regulation in the pathogenesis of inflammatory skin diseases has received more attention. Psoriasis is a common disease caused by defects in the epigenetic regulation system [82]. In particular, epigenetic changes in psoriasis and atopic dermatitis along with DNA methylation have been increasingly studied to date [83]. In psoriasis and atopic dermatitis, epigenetic changes contribute to key pathogenic events such as immune system activation, T-cell polarization, and keratinocyte dysfunction [84]. This evidence provides new perspectives on the treatment of skin diseases. It is worth considering that, unlike genetics, epigenetic changes are easily modifiable and potentially reversible.

For example, psoriasis is a multifactorial chronic inflammatory skin disease characterized by abnormal proliferation and differentiation of keratinocytes [85,86]. The results of studies show DNMT1 upregulation in peripheral blood mononuclear cells (PBMC) of psoriasis patients [87]. Evidence of altered histones in the skin of people with psoriasis has also been observed in different studies. For example, H3K27me3 marking and EZH2 levels were higher in psoriasis skin. According to research knocking out EZH2 in the epidermis of mice inhibits cell proliferation, so it is not surprising that increased levels of EZH2 in the epidermis are associated with higher and aberrant proliferation of keratinocytes, leading to psoriatic

hyperplasia [88]. However, Pharmacological inhibition of EZH2 decreases H3K27me3 levels and cell proliferation [89].

Atopic dermatitis is a heterogeneous disease in which mutations in genes encoding epidermal structural proteins, suppressor enzymes, and their inhibitors play a role in their pathogenesis [90]. The role of regulator genes of innate or adaptive immune responses and environmental factors inducing disease have also been added to this field. Related recent studies have pointed to the key role of epigenetic changes in disease development [91,92]. Documentation shows that the epigenetic profile in patients with atopic dermatitis (AD) is different from that observed in healthy individuals. This profile changes in genes that influence the regulation of the immune response and inflammatory processes, e.g., genes that influence both T_H1 orientation and promote T_H2 responses, innate immune genes, and genes that encode epidermal structural proteins [92].

4. Limitations and Risks

Ethics and safety concerns surrounding CRISPR gene editing have been raised all over the world. Many scientists agree that there is still much work to be done to improve accuracy and ensure that changes made to one part of the genome do not have unpredicted consequences, especially in the application towards human trials [93].

The CRISPRa and CRISPRi gene regulation techniques produce temporary modifications in the gene. This transience does not pose a problem in postmitotic cells or disease indications where temporary gene expression yields a therapeutic benefit. However, some illnesses require long-lasting modifications in their impaired genes. Gene expression is locally regulated by epigenetic changes as mentioned before. Epigenetic changes, such as adding methyl or acetyl groups on histone residues are frequently durable and can be passed down to newly divided cells [94]. On this account, to effect chemical alterations to DNA or chromatin, many epigenetic modifiers have been fused to CRISPR proteins [95]. To overcome this challenge, CRISPRoff and CRISPR-KAL can silence a gene for a prolonged period of time (for instance, several months) by altering H3K9me3 and DNA methylation [96,97]. These methods might work well for treating conditions that necessitate ongoing gene manipulation. However, the clinical application of epigenome engineering, like genome editing, is limited by targeted delivery methods and possible off-targeting effects.

Regarding some diseases like facioscapulohumeral muscular dystrophy, which is caused by overexpression of DUX4 gene, CRISPR/Cas9 gene editing system is literally inefficient. High number of genomic copies and DSBs that Cas9 must cause in this region, activate apoptosis. Therefore, dCas9 has been proven that can reduce the expression of DUX4 in vivo and in vitro without any cell apoptosis [98].

Delivery system is the major challenge toward applying CRISPR-based tools. However, there are controllable and efficient delivery methods for ex vivo experiments, such as microinjection and electroporation, many gene therapies require in vivo deliveries [99]. AAV and adenoviral vectors have been used to deliver CRISPR cargos for in vivo trials [100]. Limited packaging capacity of viral vectors are their negative feature as an efficient delivery system. Furthermore, Lipid nano particles have the ability to deliver CRISPR tools as RNA with greater efficiencies and lower off-targets than viral vectors. However, most of these nanoparticles will traffic to the liver and cannot reach their destinated tissue [101]. Therefore, CRISPR tools are restricted

by an efficient delivery system that is able to get their large cargos to their targeted tissues with the lowest toxicity and highest efficiency and specificity.

5. Conclusion

By redesigning the CRISPR-Cas9 system, the application of epigenetic editing has become more feasible. Numerous factors influence the efficiency of epigenetic editing, such as the targeted tissue, the chromatin context of the therapeutic gene of interest, and the epigenome editor's copy number. Many significant factors still limit the development of epigenome engineering, preventing it from being used in humans. Nevertheless, epigenetic editing remains a useful tool for altering transcription in comparison to gene editing tools, which change the sequence of genes permanently. These state-of-art technologies have the capacity to be employed in various human tissues to prevent many environmental and congenital disorders. In this regard, skin disorders are among the candidate disorders that can be precisely cured by manipulating the human epigenome. A more concise understanding of factors that regulate gene expression by chromatin remodeling and epigenetic changes can help us to treat skin diseases and even rejuvenate aged keratinocytes in adults.

Author contributions

A.S.S., M.B., and M.R. conceptualized the idea for the article and wrote the manuscript. Y.N. illustrated the figures. J.F. supervised and reviewed the final draft. All authors read and approved the final manuscript.

References

1. Cavalli G, Heard E. Advances in epigenetics link genetics to the environment and disease. Nature. 2019;571:489-99.

2. Klemm SL, Shipony Z, Greenleaf WJ. Chromatin accessibility and the regulatory epigenome. Nat Rev Genet. 2019;20:207-20.

3. Du J, Johnson LM, Jacobsen SE, et al. DNA methylation pathways and their crosstalk with histone methylation. Nat Rev Mol Cell Biol. 2015;16:519-32.

4. Shema E, Bernstein BE, Buenrostro JD. Single-cell and single-molecule epigenomics to uncover genome regulation at unprecedented resolution. Nat Genet. 2019;51:19-25.

5. Kundaje A, Meuleman W, Ernst J, et al. Integrative analysis of 111 reference human epigenomes. Nature. 2015;518:317-30.

6. Nakamura M, Gao Y, Dominguez AA, et al. CRISPR technologies for precise epigenome editing. Nat Cell Biol. 2021;23:11-22.

7. Akhtar W, de Jong J, Pindyurin AV, et al. Chromatin position effects assayed by thousands of reporters integrated in parallel. Cell. 2013;154:914-27.

8. Moore JE, Purcaro MJ, Pratt HE, et al. Expanded encyclopaedias of DNA elements in the human and mouse genomes. Nature. 2020;583:699-710.

9. Stricker SH, Köferle A, Beck S. From profiles to function in epigenomics. Nat Rev Genet. 2017;18:51-66.

10. Holtzman L, Gersbach CA. Editing the Epigenome: reshaping the genomic landscape. Annu Rev Genomics Hum Genet. 2018;19:43-71.

11. Hathaway NA, Bell O, Hodges C, et al. Dynamics and memory of heterochromatin in living cells. Cell. 2012;149:1447-60.

12. Botchkarev VA, Gdula MR, Mardaryev AN, et al. Epigenetic regulation of gene expression in keratinocytes. J Invest Dermatol. 2012;132:2505-21.

13. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346:1258096.

14. Saber Sichani A, Ranjbar M, Baneshi M, et al. A review on advanced CRISPR-based genome-editing tools: base editing and prime editing. Mol Biotechnol. 2023;65:849-60.

15. Chen PJ, Liu DR. Prime editing for precise and highly versatile genome manipulation. Nat Rev Genet. 2023;24:161-77.

16. Christie KA, Guo JA, Silverstein RA, et al. Precise DNA cleavage using CRISPR-SpRYgests. Nat Biotechnol. 2023;41:409-16.

17. Qi LS, Larson MH, Gilbert LA, et al. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. Cell. 2013;152:1173-83.

18. Ricci R, Colasante G. CRISPR/dCas9 as a therapeutic approach for neurodevelopmental disorders: innovations and limitations compared to traditional strategies. Dev Neurosci. 2021;43:253-61.

19. Chavez M, Chen X, Finn PB, et al. Advances in CRISPR therapeutics. Nat Rev Nephrol. 2023;19:9-22.

20. Choudhury SR, Cui Y, Lubecka K, et al. CRISPR-dCas9 mediated TET1 targeting for selective DNA demethylation at BRCA1 promoter. Oncotarget. 2016;7:46545-56.

21. Goell JH, Hilton IB. CRISPR/Cas-based epigenome editing: advances, applications, and clinical utility. Trends Biotechnol. 2021;39:678-91.

22. Sternberg SH, Doudna JA. Expanding the Biologist's Toolkit with CRISPR-Cas9. Mol Cell. 2015;58:568-74.

23. Zhao W, Wang Y, Liang FS. Chemical and light inducible epigenome editing. Int J Mol Sci. 2020;21:998.

24. Rots MG, Jeltsch A. Editing the epigenome: overview, open questions, and directions of future development. Methods Mol Biol. 2018;1767:3-18.

25. Cano-Rodriguez D, Gjaltema RA, Jilderda LJ, et al. Writing of H3K4Me3 overcomes epigenetic silencing in a sustained but context-dependent manner. Nat Commun. 2016;7:12284.

26. Willyard C. The epigenome editors: how tools such as CRISPR offer new details about epigenetics. Nat Med. 2017;23:900-3.

27. Mazzone R, Zwergel C, Artico M, et al. The emerging role of epigenetics in human autoimmune disorders. Clin Epigenetics. 2019;11:34.

28. Ray D, Yung R. Immune senescence, epigenetics and autoimmunity. Clin Immunol. 2018;196:59-63.

29. Xu H, Yu H, Jin R, et al. Genetic and Epigenetic targeting therapy for pediatric acute lymphoblastic leukemia. Cells. 2021;10:3349.

30. Orioli D, Dellambra E. Epigenetic regulation of skin cells in natural aging and premature aging diseases. Cells. 2018;7:268.

31. Dehkordi AN, Babaheydari FM, Chehelgerdi M, et al. Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. Stem Cell Res Ther. 2019;10:111.

32. Botchkarev VA, Gdula MR, Mardaryev AN, et al. Epigenetic regulation of gene expression in keratinocytes. J Invest Dermatol. 2012;132:2505-21.

33. Avgustinova A, Benitah SA. Epigenetic control of adult stem cell function. Nat Rev Mol Cell Biol. 2016;17:643-58.

34. Gökbuget D, Blelloch R. Epigenetic control of transcriptional regulation in pluripotency and early differentiation. Development. 2019;146:dev164772.

35. Dompe C, Janowicz K, Hutchings G, et al. Epigenetic research in stem cell bioengineering-anti-cancer therapy, regenerative and reconstructive medicine in human clinical trials. Cancers. 2020;12:1016.

36. Braun SMG, Kirkland JG, Chory EJ, et al. Rapid and reversible epigenome editing by endogenous chromatin regulators. Nat Commun. 2017;8:560.

37. Pei WD, Zhang Y, Yin TL, et al. Epigenome editing by CRISPR/Cas9 in clinical settings: possibilities and challenges. Brief Funct Genomics. 2019;19:215-28.

38. Komor AC, Badran AH, Liu DR. CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell. 2017;168:20-36.

39. Larson MH, Gilbert LA, Wang X, et al. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. Nat Protoc. 2013;8:2180-96.

40. Chavez A, Tuttle M, Pruitt BW, et al. Comparison of Cas9 activators in multiple species. Nat methods. 2016;13:563-7.

41. Pengue G, Lania L. Krüppel-associated box-mediated repression of RNA polymerase II promoters is influenced by the arrangement of basal promoter elements. Proc Natl Acad Sci USA. 1996;93:1015-20.

42. Pei WD, Zhang Y, Yin TL, eta al. Epigenome editing by CRISPR/Cas9 in clinical settings: possibilities and challenges. Brief Funct Genomics. 2020;19:215-28.

43. Groner AC, Meylan S, Ciuffi A, et al. KRAB-zinc finger proteins and KAP1 can mediate long-range transcriptional repression through heterochromatin spreading. PLoS Genet. 2010;6:e1000869.

44. Gardiner-Garden M, Frommer M. CpG islands in vertebrate genomes. J Mol Biol. 1987;196:261-82.

45. Chedin F, Lieber MR, Hsieh CL. The DNA methyltransferase-like protein DNMT3L stimulates de novo methylation by Dnmt3a. Proc Natl Acad Sci USA. 2002;99:16916-21.

46. Lei Y, Zhang X, Su J, et al. Targeted DNA methylation in vivo using an engineered dCas9-MQ1 fusion protein. Nat Commun. 2017;8:16026.

47. Wu H, Zhang Y. Reversing DNA methylation: mechanisms, genomics, and biological functions. Cell. 2014;156:45-68.

48. Rasmussen KD, Helin K. Role of TET enzymes in DNA methylation, development, and cancer. Genes Dev. 2016;30:733-50.

49. Wang H, Guo R, Du Z, et al. Epigenetic targeting of granulin in hepatoma cells by synthetic CRISPR dCas9 epi-suppressors. Mol Ther Nucleic Acids. 2018;11:23-33.

50. Pulecio J, Verma N, Mejía-Ramírez E, et al. CRISPR/Cas9-based engineering of the epigenome. Cell Stem Cell. 2017;21:431-47.

51. Zhu D, Li X, Tian Y. Mitochondrial-to-nuclear communication in aging: an epigenetic perspective. Trends Biochem Sci. 2022;47:645-59.

52. Sharma N, Pasala MS, Prakash A. Mitochondrial DNA: epigenetics and environment. Environ Mol Mutagen. 2019;60:668-82.

53. Chavez A, Tuttle M, Pruitt BW, et al. Comparison of Cas9 activators in multiple species. Nat Methods. 2016;13:563-7.

54. Li K, Liu Y, Cao H, et al. Interrogation of enhancer function by enhancer-targeting CRISPR epigenetic editing. Nat Commun. 2020;11:485.

55. Baumann V, Wiesbeck M, Breunig CT, et al. Targeted removal of epigenetic barriers during transcriptional reprogramming. Nat Commun. 2019;10:2119.

56. Braun SMG, Kirkland JG, Chory EJ, et al. Rapid and reversible epigenome editing by endogenous chromatin regulators. Nat Commun. 2017;8:560.

57. Cheng AW, Jillette N, Lee P, et al. Casilio: a versatile CRISPR-Cas9-Pumilio hybrid for gene regulation and genomic labeling. Cell Res. 2016;26:254-7.

58. Cao J, Yan Q. Histone ubiquitination and deubiquitination in transcription, DNA damage response, and cancer. Front Oncol. 2012;2:26.

59. Tan M, Luo H, Lee S, et al. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell. 2011;146:1016-28.

60. Fischle W, Tseng BS, Dormann HL, et al. Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation. Nature. 2005;438:1116-22.

61. Kebede AF, Nieborak A, Shahidian LZ, et al. Histone propionylation is a mark of active chromatin. Nat Struct Mol Biol. 2017;24:1048-56.

62. Kweon SM, Chen Y, Moon E, et al. An adversarial DNA N(6)-methyladenine-sensor network preserves polycomb silencing. Mol Cell. 2019;74:1138-47.e6.

63. Hilton IB, D'Ippolito AM, Vockley CM, et al. Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. Nat Biotechnol. 2015;33:510-7.

64. El-Serafi AT, El-Serafi I, Steinvall I, et al. A systematic review of keratinocyte secretions: a regenerative perspective. Int J Mol Sci. 2022;23:7934.

65. Min M, Chen XB, Wang P, et al. Role of keratin 24 in human epidermal keratinocytes. PloS One. 2017;12:e0174626.

66. Kumari S, Pasparakis M. Epithelial cell death and inflammation in skin. Curr Top Microbiol Immunol. 2017;403:77-93.

67. Leśniak W. Epigenetic regulation of epidermal differentiation. Epigenomes. 2021;5:1.

68. Simpson CL, Patel DM, Green KJ. Deconstructing the skin: cytoarchitectural determinants of epidermal morphogenesis. Nat Rev Mol Cell Biol. 2011;12:565-80.

69. Kardeh S, Saber A, Mazloomrezaei M, et al. Telomere targeting is insufficient to ameliorate multifaceted hallmarks of aging in cultured keratinocytes. Burns. 2022;48:470-1.

70. Holliday R, Pugh JE. DNA modification mechanisms and gene activity during development. Science. 1975;187:226-32.

71. Moltrasio C, Romagnuolo M, Marzano AV. Epigenetic mechanisms of epidermal differentiation. Int J Mol Sci. 2022;23:4874.

72. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: basic mechanisms and role in cardiovascular disease. Circ. 2011;123:2145-56.

73. Huang B, Jiang C, Zhang R. Epigenetics: the language of the cell? Epigenomics. 2014;6:73-88.

74. Li J, Xu X, Tiwari M, et al. SPT6 promotes epidermal differentiation and blockade of an intestinal-like phenotype through control of transcriptional elongation. Nat Commun. 2021;12:784.

75. Sethi I, Gluck C, Zhou H, et al. Evolutionary re-wiring of p63 and the epigenomic regulatory landscape in keratinocytes and its potential implications on species-specific gene expression and phenotypes. Nucleic Acids Res. 2017;45:8208-24.

76. Li J, Jiang TX, Hughes MW, et al. Progressive alopecia reveals decreasing stem cell activation probability during aging of mice with epidermal deletion of DNA methyltransferase 1. J Invest Dermatol. 2012;132:2681-90.

77. Driskell I, Oda H, Blanco S, et al. The histone methyltransferase Setd8 acts in concert with c-Myc and is required to maintain skin. EMBO J. 2012;31:616-29.

78. Myers JA, Couch T, Murphy Z, et al. The histone methyltransferase Setd8 alters the chromatin landscape and regulates the expression of key transcription factors during erythroid differentiation. Epigenetics Chromatin. 2020;13:16.

79. Brown S, Worthy SA, Langtry JAA, et al. Tracking tumor kinetics in patients with germline CYLD mutations. J Am Acad Dermatol. 2018;79:949-51.

80. Li X, Xu F, Zhang Z, et al. Dynamics of epigenetic regulator gene BCOR mutation and response predictive value for hypomethylating agents in patients with myelodysplastic syndrome. Clin Epigenetics. 2021;13:169.

81. Nagy N, Dubois A, Szell M, et al. Genetic testing in CYLD cutaneous syndrome: an update. Appl Clin Genet. 2021;14:427-44.

82. Chandra A, Senapati S, Roy S, et al. Epigenome-wide DNA methylation regulates cardinal pathological features of psoriasis. Clin Epigenetics. 2018;10:108.

83. Nedoszytko B, Szczerkowska-Dobosz A, Stawczyk-Macieja M, et al. Pathogenesis of psoriasis in the "omic" era. part II. genetic, genomic and epigenetic changes in psoriasis. Postepy Dermatol Alergol. 2020;37:283-98.

84. Yi JZ, McGee JS. Epigenetic-modifying therapies: An emerging avenue for the treatment of inflammatory skin diseases. Exp Dermatol. 2021;30:1167-76.

85. Ni X, Lai Y. Keratinocyte: a trigger or an executor of psoriasis? J Leukoc Biol. 2020;108:485-91.

86. Rendon A, Schäkel K. Psoriasis pathogenesis and treatment. Int J Mol Sci. 2019;20:1475.

87. Zhang P, Su Y, Chen H, et al. Abnormal DNA methylation in skin lesions and PBMCs of patients with psoriasis vulgaris. J Dermatol Sci. 2010;60:40-2.

88. Zhang T, Yang L, Ke Y, et al. EZH2-dependent epigenetic modulation of histone H3 lysine-27 contributes to psoriasis by promoting keratinocyte proliferation. Cell Death Dis. 2020;11:826.

89. Xu L, Tang H, Wang K, et al. Pharmacological inhibition of EZH2 combined with DNA-damaging agents interferes with the DNA damage response in MM cells. Mol Med Rep. 2019;19:4249-55.

90. Al-Shobaili HA, Ahmed AA, Alnomair N, et al. Molecular genetic of atopic dermatitis: an update. Int J Health Sci (Qassim). 2016;10:96-120.

91. Moosavi A, Motevalizadeh Ardekani A. Role of Epigenetics in Biology and Human Diseases. Iran Biomed J. 2016;20:246-58.

92. Nedoszytko B, Reszka E, Gutowska-Owsiak D, et al. Genetic and epigenetic aspects of atopic dermatitis. Int J Mol Sci. 2020;21:6484.

93. Kotagama OW, Jayasinghe CD, Abeysinghe T. Era of genomic medicine: a narrative review on CRISPR technology as a potential therapeutic tool for human diseases. Biomed Res Int. 2019;2019:1369682.

94. Lawrence M, Daujat S, Schneider R. Lateral thinking: how histone modifications regulate gene expression. Trends Genet. 2016;32:42-56.

95. Nakamura M, Gao Y, Dominguez AA, et al. CRISPR technologies for precise epigenome editing. Nat Cell Biol. 2021;23:11-22.

96. Nuñez JK, Chen J, Pommier GC, et al. Genome-wide programmable transcriptional memory by CRISPR-based epigenome editing. Cell. 2021;184:2503-19.e17.

97. Nakamura M, Ivec AE, Gao Y, Qi LS. Durable CRISPR-based epigenetic silencing. BioDesign Research. 2021;2021:9815820

98. Himeda CL, Jones TI, Jones PL. CRISPR/dCas9-mediated transcriptional inhibition ameliorates the epigenetic dysregulation at D4Z4 and represses DUX4-fl in FSH muscular dystrophy. Mol. 2016;24:527-35.

99. Raguram A, Banskota S, Liu DR. Therapeutic *in vivo* delivery of gene editing agents. Cell. 2022;185:2806-27.

100. García M, Bonafont J, Martínez-Palacios J, et al. Preclinical model for phenotypic correction of dystrophic epidermolysis bullosa by in vivo CRISPR-Cas9 delivery using adenoviral vectors. Mol Ther Methods Clin Dev. 2022;27:96-108.

101. Lin Y, Wagner E, Lächelt U. Non-viral delivery of the CRISPR/Cas system: DNA versus RNA versus RNP. Biomater Sci. 2022;10:1166-92.