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Skin Rejuvenation by Modulation of DNA Methylation

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ABSTRACT

Skin aging is driven by a complex set of cellular pathways. Among these, epigenetic mechanisms have garnered particular attention, because of their sensitivity to environmental and lifestyle factors. DNA methylation represents the longest known and best understood epigenetic mechanism. We explain how DNA methylation might function as an interface between the environment and the genome of human skin. Exposures to different environmental factors and lifestyles are known to modulate age-related methylation patterns, as illustrated by their effect on DNA methylation clocks. Human skin provides a particularly well-suited tissue for understanding age-related methylation changes and it has been shown recently that modulation of DNA methylation can induce skin rejuvenation. We explain how the use of mildly demethylating agents can be safeguarded to ensure the specific removal of age-related DNA methylation changes. We also identify important areas of future research, leading to a deeper understanding of the mechanisms that drive epigenetic aging and to the development of further refined intervention strategies.

1 | Introduction

Healthy and youthful skin are of substantial importance for human well-being. This requires a better understanding about how physical and social environments impact health and the recommendations for healthy lifestyles including balanced diet, exercise, avoidance of stress, alcohol, tobacco smoke and excessive sun exposure. In addition, scientific concepts have emerged that explain the interaction between the environment and the genome and that provide opportunities for the development of functional rejuvenation strategies. One such concept is provided by the research field of epigenetics, where DNA methylation acts as a mechanism that allows cells to adapt to changing environments. We describe dynamic DNA methylation changes that are part of the communication between tissues and their environment and that can be utilised as a performance indicator. We further show that human skin is particularly suited for the analysis of environment-related epigenetic changes, especially in the aging context. Skin also provides unique opportunities for the development of innovative epigenetic diagnostic and therapeutic approaches that promote skin rejuvenation. Finally, we summarise the evidence supporting that targeting of the DNMT1 DNA methyltransferase can be used to restore youthful DNA methylation patterns, resulting in an improved skin homeostasis. We also suggest future directions that could help to improve and refine these approaches.

2 | DNA Methylation as a Key Epigenetic Regulatory Mechanism

Epigenetics describes the layer of information above ('epi') the DNA sequence. Historically, epigenetics emanated from developmental biology to define an interface between the genotype and the phenotype [1]. DNA methylation represents the longest known and best understood epigenetic mark. In mammals, the vast majority of DNA methylation marks are found at the carbon-5 position of cytosine residues and in a CpG sequence

context. This covalent modification of DNA is catalysed by a conserved class of DNA methyltransferases (DNMTs), which cooperate to establish and maintain DNA methylation patterns during embryonic development and tissue homeostasis [2]. Broadly, DNA methylation patterns are established by DNMT3 enzymes during early embryogenesis, and then maintained by DNMT1, which copies methylation patterns from the parental (methylated) strand to the newly synthesised strand after DNA replication [3]. In mammals, the combined activities from these enzymes result in a widely methylated genome.

DNMT activity is antagonised by the TET dioxygenases, that catalyse the oxidation of 5-methylcytosine, resulting in DNA demethylation [4]. TETs can be targeted by transcription factors and have also been shown to have highest activity at active regulatory elements [5]. Consequently, gene regulatory elements are regions of particularly high DNA methylation dynamics [6]. Gene regulatory elements are highly enriched for transcription factor binding sites and DNA methylation has been shown to affect transcription factor binding, both positively and negatively [7]. This provides a mechanistic explanation for how DNA methylation modulates gene expression [6]. A prominent example for this effect is provided by the well-known hypermethylation of CpG island-associated promoters, which is strongly associated with gene silencing [8].

3 | Environmental Impacts on DNA Methylation Patterns

Environmental factors have long been considered to modulate epigenetic modifications, including DNA methylation [9]. Supporting evidence has been provided for a number of environmental factors, including smoking, diet and exercise [10–12]. Although effect sizes often appear relatively small, these findings are consistent with the general concept that epigenetic mechanisms allow rapid responses to changing environments [13]. However, it is also important to notice that the mammalian epigenome is generally considered as relatively stable [14], consistent with the notion that core epigenetic functions, such as cell-type specification should be robust against environmental influences.

The arguably most compelling evidence for environmental epigenetic modulation comes from the paradigmatic Agouti viable yellow (A^{vy}) and Axin Fused ($Axin^{Fu}$) loci in the mouse. These loci are considered metastable epialleles that result in quantifiable phenotypic variability in coat colour and tail morphology, respectively. Phenotypic variability is linked to the insertion of a transposable (IAP) element into the Agouti or the Fused loci and correlates with the level of DNA methylation at the IAP element, and with corresponding changes in the expression of the Agouti and Axin genes [15, 16]. Notably, it has been shown that these alleles can be modified by dietary supplements, such as folic acid, vitamin B12, choline, and betaine [17, 18]. Furthermore, more recently published data suggest that variable methylation of promoter-associated IAP elements represents a generalisable mechanism to explain epigenetic variation in the mouse [19]. Interestingly, while the methylation state of individual metastable epialleles was found to be locus-specific within an individual, it was lost through germline passage, which is consistent with the notion that acquired phenotypes are usually not heritable in mammals [14]. Transposable elements represent a considerable proportion of the mammalian genome. While most of these elements are silenced by CpG methylation, a subset can exist in metastable states and affect the activity of neighbouring genes. These variably methylated transposable elements appear to be particularly sensitive to environmental factors and have also been recognised as biosensors for environmentally induced epigenetic alterations [20].

Altogether, these findings are consistent with the notion that the human epigenome is not homogeneous, but rather organised in multiple layers (Figure 1). The inner layer forms the stable 'core' of the epigenome, which comprises promoters and gene bodies, and defines cellular identity. The surrounding 'mantle' of the epigenome mediates cellular plasticity and comprises dynamically methylated gene regulatory elements, such as enhancers. The outer 'shell' of the epigenome mediates phenotypic variability and includes transposable-element associated metastable epialleles. Because of their lesser role in cellular viability and functionality, it is reasonable to assume that these outer layers are less protected by safeguarding mechanisms, such as neighbour-guided error correction [21]. This permits the accumulation of epigenetic variants.

4 | Age-Related DNA Methylation Changes and DNA Methylation Clocks

Age-related DNA methylation changes, resulting from intrinsic aging processes and the life-long exposure to different environmental conditions, have been described in many human tissues and contexts [22] and represent valuable biomarkers for aging. This is best illustrated by their age-predicting ability and the development of DNA methylation clocks as composite biomarkers for human aging [23]. For example, the widely known 'Horvath clock' is based on the methylation levels of 353 CpG sites across the human genome that were identified by regressing chronological age on roughly 27 000 DNA methylation marks [24]. Notably, this clock has a prediction accuracy of 3.6 years across many tissues and cell types, which equals or exceeds the accuracy of established forensic age prediction methods [24].

It seems plausible to assume that a closer analysis of DNA methylation clocks would provide novel opportunities to better understand the molecular biology of aging. However, a pathway analysis of the genes that co-localise with the 353 Horvath clock CpGs did not prove to be particularly insightful, showing enrichment for cell death/survival, cellular growth/proliferation, organismal/tissue development, and cancer [24]. Alternatively, it has been suggested that the progressive DNA methylation changes that are captured by the clock primarily reflect a genome-wide decay or smoothening of the aging epigenetic landscape [25]. Intriguingly, a recent study has provided compelling evidence that a significant number of clock CpGs reflects the ratio of naive to activated T-cells [26]. Aging tissues are increasingly invaded by activated T-cells. Furthermore, activated T-cells had a DNA methylation age that was up to 40 years higher compared to naive T-cells from the same donor [26]. The age-related invasion of epigenetically older, activated T-cells therefore represents an interesting example for how altered

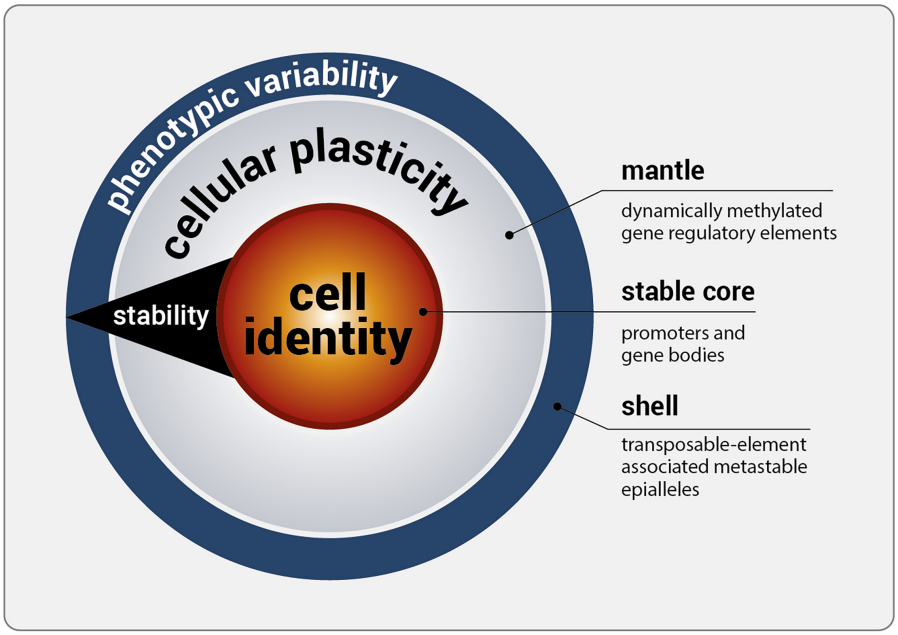

FIGURE 1 | The human DNA methylome is organised in multiple layers. An inner 'core' comprises promoters and gene bodies and defines cellular identity. The surrounding 'mantle' mediates cellular plasticity and comprises dynamically methylated gene regulatory elements, such as enhancers. The outer 'shell' mediates phenotypic variability and includes transposable-element associated metastable epialleles.

tissue composition, rather than epigenetic reprogramming, underpins observed changes in DNA methylation patterns. Systematic approaches to analyse and dissect the mechanisms that drive DNA methylation clocks have been established and hold significant promise for improving our understanding of human aging [27–29].

Differences between DNA methylation age and chronological age have repeatedly been interpreted to be reflective of the general health status [23]. DNA methylation age acceleration is associated with large number of pathologies, while DNA methylation age deceleration is associated with overall health [23]. Interestingly, a small ($N = 43$) randomised controlled trial found that an 8-weak treatment program based on diet, sleep, exercise, and relaxation patterns resulted in a 3-years decrease in DNA methylation age [30]. This suggests that DNA methylation age can be reduced by healthy diet and lifestyle choices. It should be noted that the flexibility of the clock framework allows many adaptations for specific purposes and thus provides a powerful tool to define surrogate endpoints for anti-aging strategies.

5 | Modulation of Age-Related DNA Methylation Changes

Human skin represents a paradigmatic tissue for environmental and age-driven epigenetic analyses: (1) It is characterised by a high degree of cellular homogeneity, with keratinocytes and fibroblasts being the main cell types in its main layers, respectively. (2) The differentiated epithelium is continuously renewed within about 35 to 40 days [31]. (3) Skin has a well-defined aging phenotype including measurable and visible manifestations of aging such as wrinkles, age spots, and loss of elasticity. (4) Skin is the largest human organ, and the most environmentally exposed tissue of the human body and thus exposed to various stressors, including climate conditions, ultraviolet (UV) radiation and pollutants, but also chemicals, microbes or mechanical disruption.

Age-associated epigenetic changes represent an important example that links environmental exposures to the skin epigenome (Figure 2). In a pioneering study, we provided the first description of age-related DNA methylation changes in human skin [32]. A subsequent comparison of single-base resolution epidermis methylomes revealed age-related DNA methylation changes in gene regulatory regions [33]. This provided a mechanistic explanation for how altered DNA methylation in old skin could affect skin phenotypes. In further work, we characterised epigenetic drift, i.e., the accumulation of spurious errors in DNA methylation patterning, in the human epidermis [34]. More specifically, age-related erosion of DNA methylation patterns that is characterised by a reduced dynamic range and increased heterogeneity of global methylation patterns and results in a reduced connectivity of transcriptional networks. We also showed that age-related methylation changes in the human epidermis can be used to predict the chronological age of sample donors, thus establishing the first skin DNA methylation clock [34]. Subsequent work showed that DNA methylation clocks could be developed to predict skin phenotypes [35]. More specifically, these clocks accurately predicted the skin aging phenotype represented by wrinkle grade, visual facial age, and visual age progression, respectively [35]. Together, these studies comprehensively illustrate how age-related changes in DNA methylation might connect environmental exposures to biological skin aging (Figure 2).

A role for altered DNA methylation in skin aging is also supported on the functional level, as all three active DNA methyltransferases have been linked to epidermal homeostasis [36]. However,

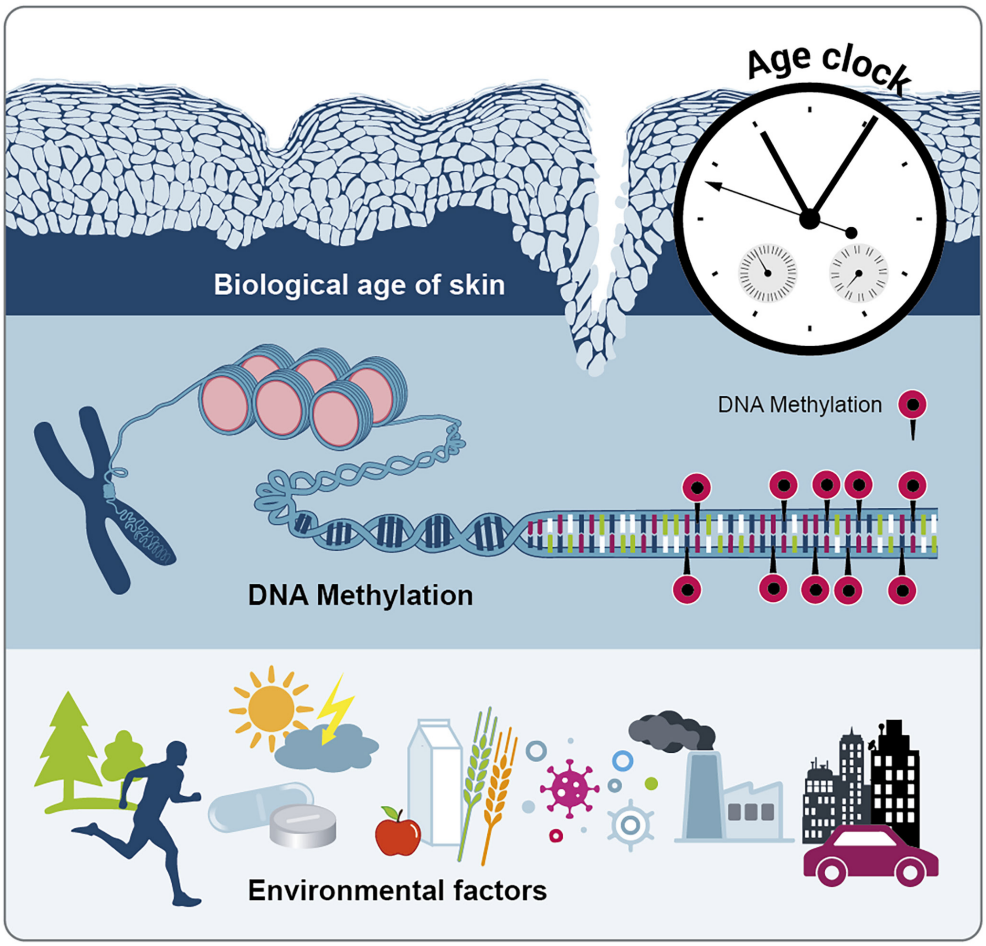

FIGURE 2 | Skin aging is driven by environmental factors. Various environmental factors, such as lifestyle and UV exposure are associated with altered DNA methylation patterns. This might cause an acceleration of biological skin aging, which is captured by DNA methylation clocks.

the de novo methyltransferases DNMT3A and DNMT3B are primarily considered as stem cell genes that establish DNA methylation patterns on unmethylated DNA [37]. In contrast, DNMT1 has been primarily linked to the mitotic inheritance of DNA methylation patterns [38], including age-related methylation changes [39]. Taken together, this establishes DNMT1 as a *bona fide* target for the development of rejuvenating active ingredients. Historically, DNMT1 has been recognised as an anticancer target, but a functional role of the enzyme in tumour formation is poorly supported by experimental data [2].

Depletion of DNMT1 in mouse and human cells causes genetic instability and apoptosis in mouse and human cells [40, 41]. However, inhibition of the enzyme by the newly developed small-molecule DNMT1 inhibitor GSK3685032 showed good in vivo tolerability, indicating considerable safety for selective active-site inhibitors of DNMT1 [42]. In agreement with this notion, mice that carry a heterozygous null allele for Dnmt1 are viable and fertile, without any detectable phenotypic aberrations [43]. While additional work will be required to precisely define the therapeutic window for dermatological applications of DNA methylation inhibitors, these findings strongly suggest a considerable safety of moderate-strength DNMT1 inhibitors.

Intervention strategies that target age-related DNA methylation patterns have been dismissed as adding royal jelly to petroleum jelly [1]. However, a more careful analysis also provides a compelling therapeutic rationale and a therapeutic approach. It is widely accepted that age-related drift causes loss of epigenetic regulatory capacity, which affects stem cell function and thus results in reduced tissue homeostasis [44]. The application of demethylating active ingredients would predominately target the more unprotected layers of the epigenome, which contains most of the environment- and age-related methylation errors. These active ingredients are selected to cause only partial methylation inhibition and a reduced DNMT1 activity leads to a passive loss of DNA methylation during DNA replication. As DNMT1 favours certain CpG sites depending on the genetic sequence and its associated chromatin networks (Figure 3) the methylation of these CpG sites is preferentially maintained under conditions of reduced DNMT1 activity. For example, it is known that chromatin marks can direct DNMTs to specific loci [45]. Furthermore, certain chromosomal proteins, such as FBXL10 protect CpG-dense regions against DNA methylation [46]. Finally, neighbour-guided error correction [21] supports the maintenance of the ground-state DNA methylation pattern (Figure 3). Thus, it is suggested that the reduced activity of DNMT1 by partial enzyme inhibition would canalise its remaining activity to prioritise on relevant methylation sites leading to a loss of age-dependent methylation, which is not instructed by 'hardwired' cellular information. As such, partial inhibition of DNA methylation would allow a stepwise optimisation and shaping of the DNA methylation pattern

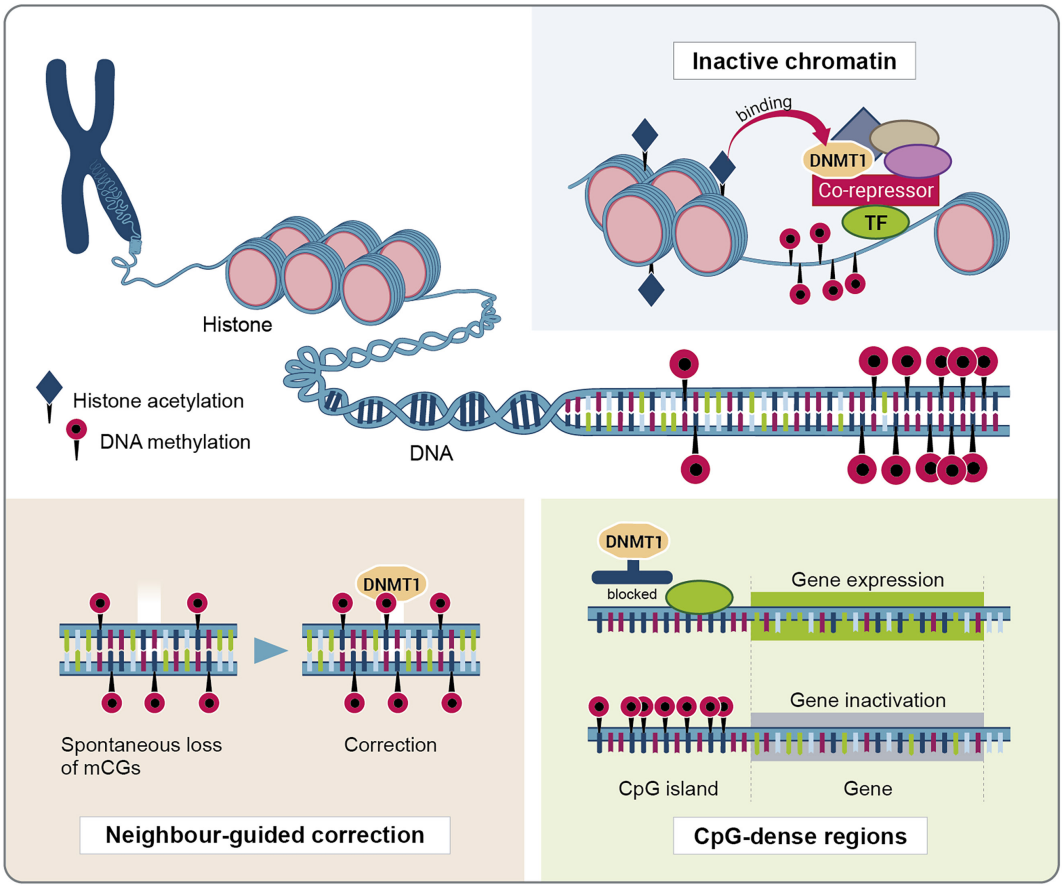

FIGURE 3 | Cellular mechanisms supporting the maintenance of the ground-state DNA methylation pattern. Certain chromatin marks are known to direct DNMTs to specific loci. In addition, chromosomal proteins can protect CpG-dense regions against DNA methylation. Finally, neighbour-guided error correction supports the maintenance of the ground-state DNA methylation pattern.

during replication over time, which finally results in the restoration of the youthful, ground-state DNA methylation pattern (Figure 4). Taken together, these findings suggest a certain selectivity in the correction of environmental- and aging-induced epigenetic errors by partial DNMT1 inhibition.

To address the need for a safe active-site DNMT1 inhibitor, we screened a library of natural substances with a DNMT1-based biochemical assay and identified dihydromyricetin (DHM) as an inhibitor [47]. DHM is considered the active ingredient of Vine tea, a health-promoting herbal tea with increasing global popularity. When added for3 days to cell culture media, DHM reduced the DNA methylation age of primary human keratinocytes by 2 years. DHM also induced noticeable rejuvenating effects in human skin models, which establishes the compound as an exciting archetype for cosmetical anti-aging applications [47]. While the long-term rejuvenation effect of DHM will have to be investigated in a future clinical study, these results establish DHM as the first epigenetic inhibitor with rejuvenating effects for human skin.

6 | Open Questions and Future Directions

Additional epigenetic mechanisms, like microRNAs and histone modifications have also been linked to aging [48, 49]. While the development of microRNA-based therapeutics remains challenging due to limitations in their specificity and delivery, highly potent and selective inhibitors have been established for histone modifying enzymes. However, these enzymes have a broad substrate specificity and modify numerous proteins which are not associated with epigenetic regulation [50]. This broad cellular mode of action has also precluded the development of predictive biomarkers so far. As such, the future improvement of epigenetic skin rejuvenation is likely to depend largely on the modulation of DNA methylation.

For the future development of epigenetic rejuvenating strategies, it will be important to build on established and safe approaches. For example, it seems reasonable to increase the potency of DHM through combinations with other epigenetically active compounds. Candidates include vitamin C, which promotes the activity of TET enzymes [51] and could thus potentiate the effects of DHM. Another vitamin, B12, has been shown to improve the in vivo efficacy of epigenetic reprogramming [52]. Vitamin B12 plays a key role in the one-carbon metabolism, which is required for methylation reactions. Furthermore, the antidiabetic substance metformin has been shown to stabilise TET2 by AMPK-dependent phosphorylation [53]. These findings suggest considerable potential for further development by combination of DNMT inhibitors with nutraceuticals and/or metabolic modulators.

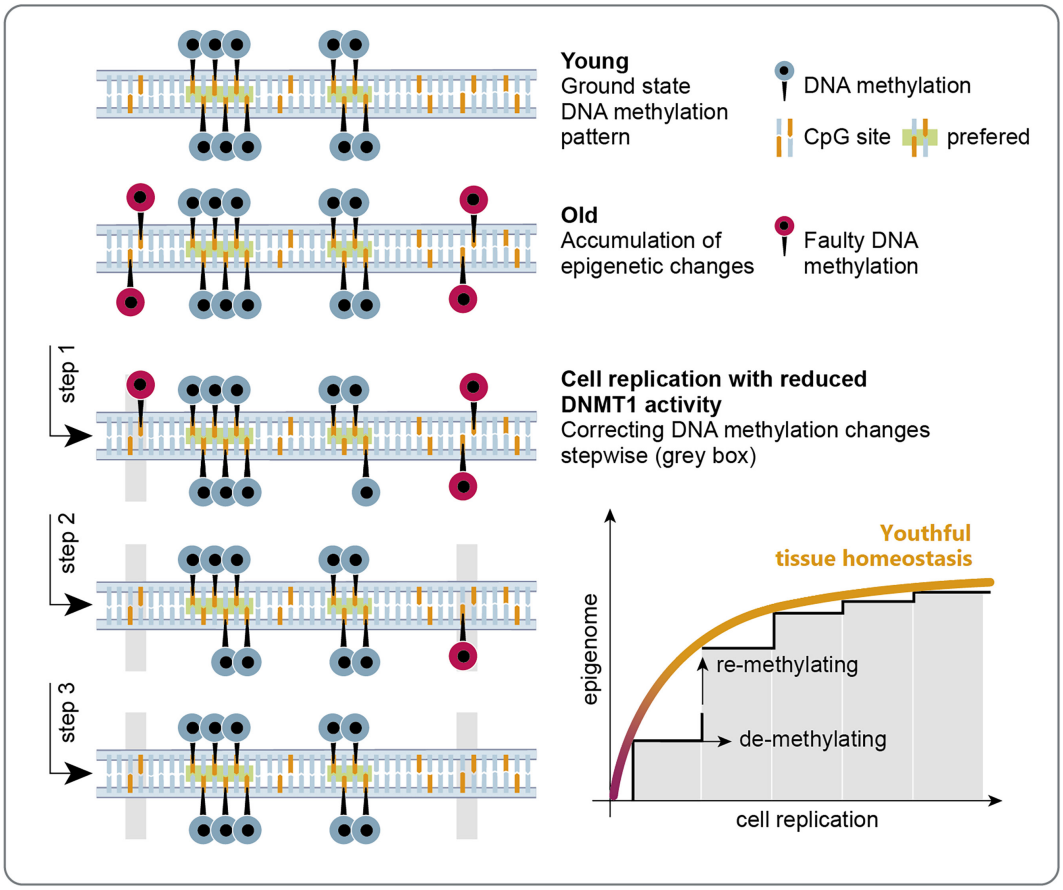

FIGURE 4 | Stepwise restoration of youthful epigenetic patterns via reduction of DNMT1 activity. Due to a reduced DNMT1 activity, ground-state methylation patterns are preferentially maintained after cell replication, while methylation patterns which are not encoded by 'hardwired' cellular information are preferentially lost over time. Continuous application of a DNMT1 inhibitor (de-methylation), in combination with cellular re-methylation can thus result in the selective erasure of age-related methylation changes.

Skin aging is accompanied by the hypermethylation of gene regulatory regions, which is associated with reduced expression of the corresponding genes [32, 33]. The restoration of the youthful epigenetic state by partial DNMT1 inhibition can result in the upregulation of age-dependently silenced genes [47]. As certain age-dependently silenced genes with relevance for proper skin homeostasis [47] are also known targets of common anti-aging treatments, like hyaluronic acid [54], it is conceivable that the re-activation of those genes by partial DNMT1 inhibition might improve the effect of common anti-aging treatments. An attractive future intervention strategy could therefore be provided by a combination of epigenetic modulators with anti-aging ingredients to potentiate the effects of established anti-aging approaches.

The clinical signs of skin aging are uniformly defined by wrinkles, uneven skin colour, loss of elasticity and loss of volume. However, skin of colour shows some unique skin aging characteristics. For example, wrinkle onset in Asian skin is delayed about 10 years compared to Caucasian skin, while pigmented spot intensity appears more prominent in Asian skin compared to Caucasian skin [55]. Furthermore, it was shown that African skin is more prone to age-related hyperpigmentation compared to Caucasian skin [56]. Notably, epidermis samples from different ethnicities also showed limited, but specific ethnic variation in their DNA methylation patterns [57]. Therefore, it could be interesting to investigate whether age-related ethnic methylation variants could be targeted to develop rejuvenation strategies that are specific for certain ethnicities. However, as the maintenance of DNA methylation patterns by DNMT1 is fundamental to all cells, it can be assumed that partial DNMT1 inhibition can restore the youthful epigenome independent from the ethnic background.

7 | Conclusions and Perspectives

In conclusion, the DNA methylome represents a well-known interface between the environment and the human genome. Environmental factors and lifestyles can result in age-related methylation changes, as illustrated by their effect on DNA methylation clocks. Human skin provides a particularly well-suited tissue for understanding age-related methylation changes and a recent study has shown that moderate inhibition of DNA methylation can induce skin rejuvenation [47]. We have now provided a mechanistic explanation how the use of mildly demethylating agents can be safeguarded to ensure the specific removal of age-related DNA methylation changes. While important points remain to be addressed, this opens an exciting new approach towards functionally rejuvenating human skin.

The acquisition of large-scale DNA methylation datasets from skin, in combination with phenotypic and health data could be used to establish correlations between the biological age and environmental or lifestyle factors. This might result in the development of novel and exciting concepts to improve rejuvenation by utilising synergies between active ingredients and healthy lifestyles. Also, advanced DNA methylation clocks can be built from such multi-dimensional datasets and then deconstructed to reveal novel molecular pathways that drive skin aging [29]. As such, epigenetics will continue to provide novel opportunities for understanding and modulating skin aging.

Author Contributions

Elke Grönniger: conception and design, manuscript writing, figure design. Heiner Max: conception and design. Frank Lyko: conception and design, manuscript writing and figure design.

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Conflicts of Interest

Elke Grönniger and Heiner Max are employees of Beiersdorf AG. Frank Lyko received consultation fees from Beiersdorf AG.

Data Availability Statement

The authors have nothing to report.

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