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# From genetics to epigenetics: new insights into keloid scarring

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## Abstract

Keloid scarring is a dermal fibroproliferative response characterized by excessive and progressive deposition of collagen; aetiology and molecular pathology underlying keloid formation and progression remain unclear. Genetic predisposition is important in the pathogenic processes of keloid formation, however, environmental factors and epigenetic mechanisms may also play pivotal roles. Epigenetic modification is a recent area of investigation in understanding the molecular pathogenesis of keloid scarring and there is increasing evidence that epigenetic changes may play a role in induction and persistent activation of fibroblasts in keloid scars. Here we have reviewed three epigenetic mechanisms: DNA methylation, histone modification and the role of non-coding RNAs. We also review the evidence that these mechanisms may play a role in keloid formation - in future, it may be possible that epigenetic markers may be used instead of prognostic or diagnostic markers here. However, there is a significant amount of work required to increase our current understanding of the role of epigenetic modification in keloid disease.

## 1 | INTRODUCTION

Keloid scarring is a dermal fibroproliferative disease characterized by excessive deposition of collagen secondary to skin damage such as trauma, burns or surgery.[1,2] The majority of keloid scars occur in the sternum handle, shoulder deltoid, jaw and ear,[3] although the reasons why keloid disease is more common at specific body sites remains unclear. Keloid scars are hard, often accompanied by itching and pain and a defining characteristic is the proliferation of the keloid scar beyond the initial boundary of the injury.[4] Relapse after surgical excision and treatment is common.[5] This disease can occur in all populations, but the incidence of keloid occurrence in the non-White race is slightly higher, and women are slightly more prone to keloid formation more than men and the incidence of the disease in young and elderly is low.[6]

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The molecular pathophysiology underlying keloid scar formation and progression is complex and poorly understood. Both genetic factors (including ethnicity) and environmental factors such as trauma are known to be important.[5,7,8] More recently, interest in the possible roles of epigenetic mechanisms in this disease has developed.

Epigenetic control of gene expression and its role in disease is an area of increasing interest and substantial complexity.[9,10] Epigenetics encompasses any mechanism of modulation of gene expression other than changes to the DNA sequence itself. Some epigenetic changes, such as DNA methylation, alter the structure of the DNA and are heritable upon cell division, providing a mechanism for sustained changes to cell phenotype.[11,12] Other epigenetic mechanisms, such as non-coding RNAs predominantly affect phenotype more transiently.[13] However, our understanding of epigenetics continues to change and in particular the dynamic changes to DNA methylation, histone acetylation and non-coding RNAs in the control of cell phenotypes. As understanding of the array of epigenetic mechanisms has increased so has the interest in investigating these mechanisms for their roles in disease. There is strong evidence that epigenetic mechanisms are important in a large number of both physiological and pathological conditions, from development to tumourigenesis.[11]

Here we have reviewed the current literature that provides evidence for a role of epigenetic changes in the pathophysiology of keloid scarring. We have focused on changes to DNA structure through methylation and histone modification as well as the role of non-coding RNAs.

## 2 | METHODS

We carried out a comprehensive search for articles related to epigenetic mechanisms in keloid scarring using databases including PubMed, Embase and China National Knowledge Infrastructure (CNKI). The key words used were epigenetics, DNA methylation, histone modification, non-coding RNA, long non-coding RNA, circular RNA, microRNA and keloid. For this review, original articles were further identified by manual searching. Articles published up to July 2016 were included in this review with the focus on key animal and human studies related to keloid scar.

## 3 | GENETICS OF KELOID SCARRING

### 3.1 | Advances in genetic studies of keloid scarring

Family survey data show that this disease may be autosomal dominant inheritance with incomplete penetrance. However, keloid scarring does not follow a simple Mendelian monogenic disease, it tends to be a polygenic disease.[14–17]

Using a genome-wide association study (GWAS) approach in the Japanese population, Nakashima M et al. identified a significant relationship between keloid scarring and four single nucleotide polymorphisms (SNPs) in three chromosomal regions: rs873549 at 1q41, rs940187 and rs1511412 at 3q22.3 and rs8032158 at 15p21.3.[18] Through using the Sequenom MassArray system, another study observed that three SNPs in two regions had significant association with keloid scarring in the Chinese Han population: rs873549 and rs1442440 at 1q41, rs2271289 at 15q21.3.[19] The identification of two common regions in these two studies in different ethnic populations strongly suggests there are common factors underpinning the pathology of keloid disease, at least in the East Asian population. Velez ED et al. conducted admixture mapping and exome genotyping. In this study, there was a significant association with keloid disease and myosin 1E (MYO1E). The study also implicated genetic elements at 15q21.2-22.3 in the pathogenesis of keloid disease in African American, Japanese and Chinese populations.[20] In a recent study, 27 SNPs and 8 disease-associated genes were screened out through whole genome sequencing in one case of Chinese Han familial keloid scarring.[21]

To date several studies have reported polymorphisms that may be associated with keloids in multiple genes with known functions relevant to fibrosis, with many genes in the transforming growth factor (TGF) pathway including TGF-β1, TGF-β2, TGF-β3, TGF-β receptor (TGF-βR)I, TGF-βRII, TGF-βRIII, mothers against decapentaplegic homologue (SMAD)3, SMAD6, SMAD7, epidermal growth factor receptor (EGFR), TNF alpha-induced protein 6 (TNFAIP6), p53 as well as human leucocyte antigen (HLA) alleles (HLA-DRB1*15, HLA-DQA1*0104, DQ-B1*0501 and DQB1*0503).[22–27]

### 3.2 | Limitations of genetic studies on keloid scarring

In recent studies, GWAS approaches have identified some susceptibility genes that promote fibroblast differentiation and activation.[8] However genetic variants only explain part of the biological or functional change. The mechanisms of triggering and maintaining the profibrotic myofibroblast phenotype are still not clear. In addition, while genetic approaches have indicated important genomic regions with keloid scarring, to date there is no clear understanding of the molecular pathogenesis of the disease. It is likely that in addition to genetic factors, there are also other important mechanisms involved in keloid disease. Environmental factors may play a role and importantly epigenetic mechanisms may also play a pivotal role.[28,29] Epigenetic modification has been shown to be an important regulator during the persistent activation process of fibroblasts in fibrotic diseases, including in pulmonary fibrosis, liver fibrosis, renal fibrosis and systemic sclerosis.[30–34] Evidence for a role of epigenetic modification in keloid disease is now also coming to light.

## 4 | EPIGENETIC MECHANISMS IN KELOID SCARRING

### 4.1 | DNA Methylation in keloid scarring

DNA methylation is the most common epigenetic modification. Methylation refers to a chemical modification process in which a methyl group from adenosyl methionine is transferred to a carbon atom in the cytosine ring of the DNA molecule. This process is catalysed

by DNA methyltransferase (DNMT) enzymes.[35,36] Methylation is most common at CpG dinucleotides.[37] The DNMT family includes three members: DNA methyltransferase 1 (DNMT1), DNA methyltransferase 3a (DNMT3a) and DNA methyltransferase 3b (DNMT3b). Although DNMT1 is involved primarily in maintaining DNA methylation status during cell division, a process known as "methylation maintenance", DNMT3a and DNMT3b both modify unmethylated DNA, generating newly methylated bases, a process known as "*de novo* methylation".[38,39]

Recent reports demonstrated that the expression of TGF-β1, phospho-smad2 and phospho-smad3 were elevated in keloid lesions, while the expression of smad7 was decreased.[40] 5-aza-2-deoxycytidine (5-aza-dC), an inhibitor of DNA methyltransferase, appeared to reverse these changes in expression level, suggesting epigenetic control was important.[40] The proportion of apoptotic cells in keloid fibroblasts in culture also can be modulated by the addition of the same methylation inhibitor, again supporting a role for methylation in important cell changes in keloid disease.[40] A study by E Y et al. investigated the expression of DNMT1 in keloid and non-keloid scar tissue and found that 100% of the keloid fibroblasts expressed DNMT1 with only 8% of normal skin fibroblasts expressing DNMT1.[41] Therefore, DNA methylation may play a vital role in the pathology of keloid scarring.

Differences in DNA methylation patterns from keloid scar and healthy tissue and cell samples have been demonstrated in a number of recent studies. Jones et al.[42–44] carried out genome-wide profiling using the Infinium HumanMethylation450 BeadChip and examined genes most differentially methylated between six keloid and six normal skin samples using a three-tiered approach. They found that there were 685 differentially methylated CpGs at Tier 3 differentially methylated CpGs screen criteria. Of this total, 190 differentially methylated promoter region CpGs corresponded to 152 unique genes. The research team further identified four hierarchical networks using Causal Network Analysis software. These relevant networks included four master regulators (pyroxamide, tributyrin, PRKG2 and PENK) and 19 intermediate regulators, strongly implicating these master regulators in the pathogenesis of keloid disease.

These studies provide support for a role of DNA methylation in keloid scarring. However, further work will be required to understand the key changes driving pathogenesis amidst the extensive changes that occur as a consequence of keloid progression.

## 4.2 | Histone modification in keloid scarring

Histone modifications are covalent modifications of N-residues in the distal amino acids, including acetylation of lysine (K) residues, ubiquitination of lysine (K) or arginine (R) residues and phosphorylation of serine (S) or threonine (T).[45,46]

Histone deacetylases (HDACs) and histone acetyltransferases (HATs) can influence gene expression *via* the removal or addition of acetyl groups to histones.[47] Previous studies have found that the use of HDAC inhibitors decreased collagen production in keloid fibroblasts *in vitro*.[48] HAT inhibition also has an anti-fibrotic effect, and overexpression of p300 (a cofactor with histone acetylase activity) had a significant pro-fibrotic effect in response to TGF-β1 in fibroblasts from scleroderma patients.[49,50]

Russell et al. have observed differential methylation of multiple genes in keloid fibroblasts with significant changes in the levels of expression of multiple fibrotic genes including IGF/IGF-binding protein 5 (IGFBP5), jagged 1 (JAG1), connective tissue growth factor (CTGF), secreted frizzled-related protein 1 (SFRP1), matrix metallopeptidase 3 (MMP3) and dermatopontin (DPT).[51] Interestingly, the use of 5-aza-dC only altered the expression levels of some of the genes of interest. Other gene expression levels, for example SFRP1, were only affected by the use of trichostatin A. This suggests both methylation and histone modification are important in the altered gene expression profiles observed in keloid fibroblasts. The altered expression levels when compared to normal skin fibroblasts were also maintained by keloid fibroblasts over the culture lifetime. This again suggests sustained modulation of gene expression and implicates epigenetic change as being important in the maintenance of the keloid fibroblast phenotype.[48,51–53]

EJ Fitzgerald O'Connor et al. investigated the expression profiles of specific HDACs in normal and keloid skin.[52] This revealed histone deacetylase 2 (HDAC2) up-regulation in human keloid tissue *in vivo*.[52] Up-regulation of HDAC2 can also be observed in scar tissue in a mouse model of wound repair.[52] Furthermore, another study found that TGF-β1 was able to trigger a concentration-dependent up-regulation of HDAC2 in murine Swiss 3T3 fibroblasts and also in cultured normal human dermal fibroblasts.[52]

This work suggests a potential use for inhibition of histone modification in keloid scarring. Further work to understand the important mechanisms and pilot clinical trials could indicate whether histone modification is a therapeutic target for the treatment of keloid scars.

## 4.3 | Non-coding RNAs in keloid scarring

Functional non-coding RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), play crucial roles in the regulation of gene expression.[54] miRNAs are a class of short, single-stranded, non-coding RNAs with a length of about 20–24 nucleotides. Through post-transcriptional regulation of gene expression, miRNAs play important roles in physiological and pathological processes.[55,56] Long non-coding RNA (lncRNA) are mRNA-like molecules, larger than 200 nucleotides, but without functional open reading frames. Emerging evidence has revealed that lncRNA plays a cis-regulatory role in gene clusters and at the entire chromosomal level.[57] Recent research has found abnormal miRNA and lncRNA expression in keloid scar cells, suggesting non-coding RNA may also play a role in keloid pathology.[58]

### 4.3.1 | miRNAs and keloid scarring

#### miRNA expression profiles in keloid scarring

Several miRNAs have been implicated in the process of fibroblast activation (Table 1). miRNA expression profiles have also been obtained from keloid tissue, fibroblasts and serum (Table 2). Using miRNA microarrays in 12 pairs of keloid tissue and corresponding normal skin,

**TABLE 1** Abnormal microRNAs (miRNAs) and the biological processes of keloid scarring

| miRNA | Regulation | Target genes | References |
|---|---|---|---|
| miR-7 | DOWN | Type I collagen | 67 |
| miR-29a | DOWN | Type I and type III collagen | 69 |
| miR-199a | DOWN | Not mentioned | 60,61 |
| miR-21 | UP | PTEN, FasL, PDCD4 | 73–75 |
| miR-196a | UP | COL1A1 and COL3A1 | 65 |
| miR-152 | UP | Unsure | 77 |
| miR-200c | DOWN | TGF-β1 | 79,80 |

Liu et al found a total of 32 differentially expressed miRNAs. Among them, 23 miRNAs were up-regulated, while 9 miRNAs were down-regulated.[59] In another study, 17 differentially expressed miRNAs were identified in four keloid tissue samples compared to three normal skin samples.[60,61] Three miRNAs in common were detected in both studies; has-miR-199a-5p, has-miR-21 and has-miR-214. miRNA expression patterns are evaluated by miRNA PCR array analysis consisting of 88 miRNAs involved in human cell differentiation and development. Sixty-five miRNAs exhibited abnormal expression in three keloid compared to three normal skin samples.[62] Interestingly, has-miRNA-21 was differentially expressed in all three studies. In the study of Guo XR et al., 12 differentially expressed miRNAs were screened in human keloids. However, the abnormal expression of has-miRNA-21 was not found.[63]

miRNA expression profiles were further compared between fibroblasts from keloid and normal skin using miRNA microarray analysis. Nine miRNAs in keloid-derived fibroblasts were different from fibroblasts of normal skin, with six miRNAs including miR-152, miR-23b-3p, miR-31-5p, miR-320c, miR-30a-5p and hsv1-miR-H7, up-regulated and three, including miR-4328, miR-145-5p and miR-143-3p, down-regulated.[64] One similar study also found 27 miRNAs were differentially expressed, 7 of which were significantly up-regulated in keloid fibroblasts, and 20 of which were significantly down-regulated.[65]

A recent study investigated the expression profiles of miRNAs in sera from nine keloid patients and seven normal controls. miRNA microarray analysis identified 37 differentially expressed miRNAs (17 up-regulated and 20 down-regulated) in keloid patients.[66] Some of the key miRNAs that appear differentially expressed in keloid cells are assessed in more detail below.

## Pivotal miRNAs in keloid scarring

**miR-7** Following on from the microarray data, Etoh M et al. confirmed the expression of miR-7 was significantly decreased in keloid tissue compared to normal skin by PCR array analysis.[67] In situ hybridization showed that the signal for miR-7 was not evident in the keloid fibroblasts but present in those from normal tissue. miR-7 has been linked to excessive collagen expression in localized scleroderma *via* the induction of an overexpression of type I collagen.[67] However, the role of miR-7 in keloid disease remains unknown.

**miR-29a** Increasing evidence has shown that miR-29 plays a central role in the progression of fibrotic disease.[68] miR-29 family expression is lower in keloid tissue when compared to normal skin tissue. In addition, levels of expression of the miR-29 family, especially miR-29a, were also significantly decreased in keloid fibroblasts compared with healthy controls.[69] miR-29 has a role in the regulation of type I and type III collagen expression.[70] Interestingly, miR-29a was markedly down-regulated in fibroblasts which were pretreated and cultured with TGF-β1, suggesting a close link between miR-29 and TGF-β1 signalling.[69] This suggests that miR-29a/TGF-β/Smad signalling pathway may be important in keloid scarring.

**miR-199a** Compared with normal skin tissue, miR-199a showed significantly lower expression in keloid tissue. The expression of miR-199a may be negatively correlated with cell proliferation.[71] Wu ZY et al. have shown that the decreased expression of miRNA-199a can influence proliferation through the regulation of the cell cycle of keloid fibroblasts.[60,61] This suggests an important role in keloid disease progression by miR-199a.

**miR-21** miR-21 is considered to be an oncogenic miRNA.[72] miR-21 is expressed at a high level in the development of fibrosis and may play a vital role in the proliferation of interstitial fibroblasts and overproduction of extracellular matrix. Liu Y et al. found that miR-21 can regulate proliferation and apoptosis through the PI3K/AKT signalling pathway and by targeting phosphatase and tensin homologue deleted on chromosome ten (PTEN) and programmed cell death 4 (PDCD4) expression in human keloid fibroblasts.[73,74] Another study confirmed the high expression levels of TGF-β1 and miR-21 in fibroblasts isolated from keloid tissue. This study further demonstrated that miR-21 can affect the expression of Fas ligand (FasL) in the presence of TGF-β1.[75] This suggests a role for miR-21 in keloid scarring.

**miR-196a** miR-196a was the first miRNA to be studied in depth. miR-196a can regulate the expression of collagen (COL1A1 and COL3A1) through its effects on the 3' untranslated region of these genes.[65,76] miR-196 has also been observed to be expressed at an elevated level in keloid cells.[65] This suggests a likely role in fibrotic disease through stabilization of elevated collagen expression.

**miR-152** Two miRNA microarray studies identified that miR-152 was significantly up-regulated in keloid fibroblasts. [59,64] Using qRT-PCR, Fang RJ et al. confirmed miR-152 increased in expression in keloid tissue. They also found miR-152 can promote keloid fibroblast proliferation and collagen synthesis.[77] Therefore, miR-152 may play a role in keloid disease. However, the specific signalling pathways that miR-152 may involve in are still unclear. Those findings highlight the need for further investigation to ascertain the roles of these molecules in keloid cells.

**miR-200c** miR-200c has been shown to be able to drive a reversal of the process of epithelial-mesenchymal transition which is induced by TGF-β1 in a variety of tumours.[78,79] A previous study has shown that the expression of miR-200c in keloid tissue is 6.92 times lower

**TABLE 2** microRNAs (miRNAs) expression profile in keloid scarring

| Sample | Number (Cases/Controls) | Differential miRNA number (up/down) | Up-regulated miRNAs | Down-regulated miRNAs | Methods | Ref. |
|---|---|---|---|---|---|---|
| Skin tissue | 4/3 | 17 (3/14) | miR-214, miR-645, miR-338-5p, miR-934, miR-199a-5p, miR-21*, miR-122*, miR-186*, miR-495, miR-412, miR-551b*, miRPlus-F1158, miRPlus-E1038, miR-1308 | miRPlus-E1106, miR-516b, miRPlus-E1247 | miRNA microarray | 60,61 |
| Fibroblast | 3/3 | 9 (3/6) | miR-152, miR-23b-3p, miR-31-5p, miR-320c, miR-30a-5p, hsv1-miR-H7 | miR-4328, miR-145-5p, miR-143-3p | miRNA microarray | 64 |
| Fibroblast | 3/3 | 27 (7/20) | miR-142-3p, miR-1249, miR-136, miR-376a*, miR-140-5p, miR-193a-3p, miR-1234 | miR-23a, miR-574-3p, let-7d, miR-331-3p, miR-24, let-7a, miR-30e*, miR-93, let-7f, miR-98, miR-31*, miR-31, miR-224, miR-30a*, miR-769-5p, miR-595, miR-196b, miR-452, miR-182, miR-196a | miRNA microarray | 65 |
| Skin tissue | 3/3 | 65 (5/60) | miR-302c, miR-127-5p, miR-21, miR-424, miR-122 | let-7a, miR-1, miR-133b, miR-206, miR-208, miR-498, miR-124, miR-129-5p, miR-130a, miR-146a, miR-182, miR-183, miR-371-3p, miR-375, miR-378, miR-92a, miR-101, miR-125a-5p, miR-128a, miR-150, miR-18b, miR-215, miR-219-5p, miR-23b, miR-26a, miR-452, miR-96, let-7d, let-7e, let-7f, let-7g, miR-100, miR-125b, miR-137, miR-141, miR-142-5p, miR-15b, miR-205, miR-20b, miR-218, miR-223, miR-302a, miR-33a, miR-520g, let-7b, miR-126, miR-16, miR-17, miR-185, miR-195, miR-20a, miR-488, miR-7, let-7c, miR-210, miR-222, miR-24, miR-301a, miR-93, miR-99a | miRNA PCR array | 62 |
| Skin tissue | 12/12 | 32 (23/9) | miR-21,miR-4269,miR-382,miR-487b,miR-155,miR-98,miR-127-3p,miR-31,miR-939,miR-134,miR-432,miR-152,miR-455-3p,miR-424,miR-379,miR-181b,miR-132,miR-494,miR-199a-5p,miR-199a-3p,miR-214,miR-3652,miR-181c | miR-203, miR-205, miR-200c, miR-200b, miR-1246, miR-222, miR-3613-3p, miR-221, miR-30b | miRNA microarray | 59 |
| Serum | 9/7 | 37 (17/20) | miR-3684*,miR-6800-3p*,miR-6796-3p*,miR-484, miR-466, miR-6891-5p, miR-1225-5p, miR-6813-3p*,miR-6805-3p*,miR-382-5p, miR-4258, miR-412-3p, miR-1185-2-3p, miR-658, miR-513a-5p, miR-195-5p, miR-6801-3p* | miR-4254,miR-6804-3p*,miR-4318*,miR-6765-5p*,miR-4317,miR-6803-3p*,miR-1470,miR-6802-3p*,miR-3151-3p, miR-6734-5p*,miR-1307-3p,miR-409-5p,miR-5196-5p*,miR-491-3p,miR-4325*,miR-4429,miR-4769-3p*,miR-4763-5p, miR-6879-5p*, miR-6836-3p | miRNA microarray | 66 |
| Skin tissue | 3/3 | 12 (1/11) | miR-1290 | miR-1204, miRPlus-F1158, miR-934, miR-186*, miR-99b*, miR-605, miR-645, miR-342-5p, miR-224*, miR-551b*, miRPlus-C1110 | miRNA microarray | 63 |

than that of normal skin tissue, suggesting that the loss of miR-200c expression may be closely related to the pathogenesis of the disease.[59] Sun et al. found that miR-200c expression significantly inhibited cell proliferation and collagen synthesis induced by TGF-β1 in keloid fibroblasts.[80] The phosphorylation of Smad2 and Smad3 was markedly reduced by exogenous miR-200c treatment. miR-200c expression has also been shown to inhibit bleomycin-induced elevation of TGF-β1 expression in keloid fibroblasts.[79,80] Therefore, there is strong evidence that the loss of miR-200c expression observed in keloid tissue may contribute to the pathology of keloid scarring.

### 4.3.2 | Long non-coding RNA (lncRNA) and keloid scarring

lncRNA can regulate gene expression and is important in the control of the cell cycle and cell proliferation.[81,82] Recently, using microarray and qRT-PCR approaches, one study identified and validated differential expression of lncRNAs in keloid tissue compared to normal skin.[83] In preliminary screening, 1731 lncRNAs were up-regulated and 782 down-regulated in expression in keloid tissue. Differential expression of mRNAs was also identified.[84] A co-expression network of lncRNA and mRNA was performed and it was found that a lncRNA, calcium voltage-gated channel subunit alpha1 G-antisense 1 (CACNA1G-AS1), was connected to a number of mRNAs that showed differential expression.

Another lncRNA, lncRNA-activated by TGF-β (lncRNA-ATB), was also observed to be up-regulated in keloid tissue. lncRNA-ATB can regulate the autocrine secretion of TGF-β2 in keloid fibroblasts by inhibiting the expression of zinc finger protein 217 (ZNF217) *via* miR-200c.[79] Therefore, these findings indicate that a lncRNA-ATB/miR-200c/ZNF217/TGF-β2 signalling axis may be involved in the initiation and progression of keloid disease.

## 5 | CONCLUSION

Epigenetics may provide a new direction for the study of the pathogenesis of keloid scarring. Expression studies have provided support for a role of epigenetic change in keloid disease, and future studies increasing sample number and study power may increase insight into the role of specific changes. There appears to be a role for multiple epigenetic mechanisms in keloid pathogenesis, including DNA methylation, histone modification and regulatory RNA changes. There is also some evidence that epigenetic modification may provide a new therapeutic option, with *in vitro* studies suggesting drugs that affect epigenetic processes can influence expression and phenotype of keloid cells. However at this stage, our understanding of the key epigenetic changes and their effects is still limited, and therefore clinical intervention will take time. Nevertheless, further work on the role of epigenetic changes in keloid scarring is warranted based on the evidence from the studies conducted to date.

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## CONFLICT OF INTEREST

The authors declare that they have no competing financial interests.

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