Table SI. Levels of evidence and key limitations of epigenetic studies in cutaneous fibrosis.

Study type/approach	Typical model (sample size)	Level of evidence[a]	Key limitations	(Ref.)
Human observational bulk omics	Human scar tissue (n=10-50)	II; translational evidence	Bulk-tissue analysis masks cellular heterogeneity; correlative; population-specific biases.	(8,33-35)
Human single-cell sequencing/spatial omics	Human tissue (3-10 samples, each comprising thousands of cells)	II-III; exploratory-translational evidence	Very small cohort sizes; high cost; technical noise; limited functional follow-up.	(28-30)
In vitro functional validation	Cultured human fibroblasts (n=3-6 biological replicates)	III; pre-clinical evidence	Simplified 2D culture lacks tissue context; potential off-target effects of transfection or editing; donor variability.	(18,36-38)
Animal model interventions	Rodent or rabbit models (n=5-10 per group)	III; pre-clinical evidence	Species differences in skin biology and healing; small sample sizes; short follow-up.	(12,36)
First-in-human interventions	Small patient cohorts (n<50)	IIa; early-phase clinical evidence	Lack of randomization or placebo in some studies; subjective or non-standardized endpoints; short duration.	(11)
Bioinformatic prediction and	Public datasets or *in silico* analysis	IV; hypothesis-generating evidence	Requires experimental validation; high false-positive rate.	(39,40)

network analysis				

[a]Level of evidence: I, large, prospective randomized controlled trials; II, smaller cohort/translational studies (IIa, with intervention; IIb, observational); III, *In vitro* or animal model studies; IV, bioinformatics/preliminary data. Classification adapted from levels for therapeutic and mechanistic evidence.

Table SII. DNA-methylation drivers of cutaneous fibrosis.

First author, year	Model/tissue (n)	Key method	Major methyl-locus targets	Direction of change	Functional read-out	Ref.
Sharma *et al*, 2016	Rabbit glaucoma surgery	5-aza-2′-deoxycytidine, sub-conj administration	Global	DNA hypomethylation (demethylation)	Collagen deposition reduced by 45% vs. vehicle control	(11)
Jones *et al*, 2015	Keloid, multi-ethnic (28)	Illumina 450K EWAS	COL1A1, COL3A1, RASAL1 and PTEN	Hypomethylation enriched in collagen-related genes; hypermethylation of anti-fibrotic genes (RASAL1, PTEN)	First keloid methylome map	(34)
Alghamdi *et al*, 2020	Keloid, recruited from Western Australia and Saudi Arabia (48)	Illumina EPIC 850K	3,214 DMCs[a]	Same directional Δβ ~0.20	Validated platform concordance	(35)
Prabsattru *et al*, 2025	Keloid, Thai (35)	Bisulfite-pyroseq	LINE-1 methylation	Δβ 0.18 vs. normal tissues	Predicts recurrence r=0.62	(36)
Stevenson *et al*, 2022	Keloid fibroblasts (12)	WGBS and RNA-seq	FOXF2 promoter	Hypermethylation results in FOXF2 downregulation	FOXF2 downregulation drives collagen upregulation	(42)

Liu *et al*, 2019	Human dermal fibroblasts under hypoxic conditions	hMeDIP-qPCR	TGF-β1 promoter	5-hydroxymethylcytosine downregulated by 40%	TGF-β1 upregulated; reversed by Vit-C	(44)
Niu and Tan, 2023	Keloid explants (8)	TET2 overexpression	COL1A2 enhancer	5-hydroxymethylcytosine upregulated	Collagen downregulated by 31%	(45)
Meevassana *et al*, 2022	Burn scars vs. normal tissue (20)	Bisulfite-PCRs	Alu and LINE-1 repeats	Hypermethylation of 8-12%	First repeat-element scan	(46)

[a]DMCs showed a 62% overlap with the results of the study reported by Jones *et al* (33). EWAS, epigenome-wide association study; DMCs, differentially methylated CpGs; WGBS, whole-genome bisulfite sequencing; hMeDIP-qPCR, hydroxymethylated DNA immunoprecipitation-quantitative PCR; Δβ, difference in methylation beta-values; RNA-seq, RNA-sequencing; 5-hydroxymethylcytosine, a stable DNA modification generated by Ten-eleven translocation-mediated oxidation of 5-methylcytosine; Vit-C, vitamin C (ascorbic acid); Alu, A short interspersed nuclear element; LINE-1, long interspersed nuclear element-1; sub-conj, subconjunctival (route of administration); COL3A1, type III collagen α1 chain; RASAL1, RAS protein activator-like 1; PTEN, phosphatase and tensin homolog.

Table SIII. Non-coding RNA networks in scar pathogenesis

First author, year	RNA class	Model/tissue (n)	Key RNAs	Expression change	Functional phenotype	Ref.
Wang *et al*, 2025	m6A-miRNA	HTSs (6)	pri-miR-31 (METTL3-mediated)	m6A hypermethylation of pri-miR-31	METTL3-mediated m6A modification of pri-miR-31 promoted mature miR-31-5p downregulation, thereby derepressing ZBTB20 to promote collagen synthesis	(13)
Li *et al*, 2015	miRNA	HTS fibroblasts (3)	miR-10a and miR-181c	Differentially expressed (miR-181c upregulated; miR-10a downregulated)	miR-181c targets uPA; miR-10a targets PAI-1; miR-181c knockdown or miR-10a overexpression increases MMP1 and decreases COL1A1	(37)
Li *et al*, 2016	miRNA	HTSs (4)	miR-21	Upregulated	miR-21 promoted TGF-β1-induced fibroproliferative expression by repressing Smad7 expression in vitro; PTEN was not investigated in this study	(38)
Jin *et al*, 2019	lncRNA	Keloid (6)	HOXA11-AS	Upregulated	Sponges miR-124-3p, leading to Smad5 upregulation	(39)
Zhang *et al*, 2020	circRNA	Keloid (6)	circPTK2 and circFNDC3B	Upregulated	Sponges miR-19a-3p and miR-29b	(40)

Mu *et al*, 2016	miRNA	HTSs (5)	miR-143-3p	Upregulated	CTGF upregulation caused an upregulation in Akt/mTOR signaling	(47)
Shimada *et al*, 2020	miRNA	SSc fibroblasts (6)	miR-29 family	Downregulated	MMP1 was upregulated; collagen-I was downregulated	(48)
Zhou *et al*, 2015	miRNA	HTSs (8)	miR-21 and miR-200b	Upregulated	Pro-fibrotic potential via TGF-β/miR-21/Smad7 and TGF-β/miR-200b/Zeb1 pathways	(49)
Liu *et al*, 2016	miRNA	HTSs (6)	miR-21	Upregulated	Smad7 signaling increased	(50)
Yan *et al*, 2016	miRNA	Keloid keratinocytes (4)	miR-21	Upregulated	Stemness and EMT	(51)
Zhou *et al*, 2017	miRNA	Keloid (7)	miR-21	Upregulated	Smad7 was downregulated; collagen was upregulated	(52)
Yan *et al*, 2020	miRNA	Keloid (5)	miR-21	Upregulated	Autophagy and migration increased	(53)
Yan *et al*, 2016	miRNA	Mouse scleroderma	miR-155	Upregulated	CTGF was upregulated; inflammation	(54)
Li *et al*, 2024	miRNA	HTSs (6)	miR-155	Upregulated	α-SMA was upregulated via AZGP1 downregulation	(55)

Gras *et al*, 2015	miRNA	HTSs (4)	miR-145	Upregulated	Myofibroblast conversion enhanced	(56)
Wang *et al*, 2017	miRNA	HTSs (5)	miR-31-5p	Upregulated	RhoA was upregulated; stress-fiber formation increased	(57)
Shi *et al*, 2018	miRNA	Keloid (6)	miR-203	Downregulated	EGR1/FGF2 were upregulated; proliferation increased	(58)
Pang *et al*, 2019	miRNA	Keloid (4)	miR-152-5p	Downregulated	Smad3 was upregulated; apoptosis decreased	(59)
Xu and Jiang, 2021	miRNA	Keloid (5)	miR-194-5p	Downregulated	NR2F2 was upregulated; fibrosis increased	(60)
Xu *et al*, 2024	exosomal miRNA	Mouse burn	miR-194	Downregulated	TGF-β1 was upregulated; fibrosis increased	(61)
Xu *et al*, 2021	lncRNA	Keloid (5)	H19	Upregulated	Sponges miR-769-5p, leading to EIF3A upregulation	(62)
Tu *et al*, 2018	lncRNA	HTSs (3)	TUG1, MALAT1	Upregulated	Pro-fibrotic effects	(63)
Li *et al*, 2021	lncRNA	HTSs (4)	LINC00173	Upregulated	β-catenin was upregulated; apoptosis decreased	(64)
Wu *et al*, 2022	lncRNA	HTSs (5)	FPASL	Downregulated	DNMT3B was upregulated; fibroblast activity increased	(65)

Li *et al*, 2021	lncRNA	HTSs (4)	COL1A2-AS1	Upregulated (induced apoptosis in normal skin fibroblasts)	p-Smad3 was repressed and β-catenin was promoted, leading to increased apoptosis	(66)
Li *et al*, 2021	lncRNA	HTSs (5)	TUG1	Upregulated	TAK1/YAP/TAZ signaling activation via miR-27b-3p sponging	(67)
Zhang *et al*, 2021	circRNA	Keloid (4)	Global circrome	N/A	91 DE-circRNAs were catalogued	(68)
Lv *et al*, 2021	circRNA	Keloid (5)	circCOL5A1	Upregulated	Sponges miR-7-5p, upregulates Epac1, and activates PI3K/Akt signaling	(69)
Zhu *et al*, 2022	circRNA	Keloid (4)	circ_0057452	Upregulated	miR-7-5 sponging increased GAB1 axis activity	(70)
Xu *et al*, 2024	m6A-circRNA	HTSs (5)	circGLIS3 (ALKBH5-mediated)	Downregulated 5hmC	Demethylation resulted in downregulation of ECM deposition	(71)

This table catalogs the diverse ncRNA species implicated in scar pathogenesis, illustrating their roles in forming interconnected regulatory circuits that sustain fibroblast activation and collagen deposition. α-SMA, α-smooth muscle actin; Akt, protein kinase B; ALKBH5, α-ketoglutarate-dependent dioxygenase alkB homolog 5; AZGP1, zinc-α-2-glycoprotein 1; circRNA, circular RNA; CTGF, connective tissue growth factor; DE, differentially expressed; DNMT3B, DNA methyltransferase 3B; ECM, extracellular matrix; EGR1, early growth response 1; EIF3A, eukaryotic translation initiation factor 3 subunit A; EMT, epithelial-mesenchymal transition; Epac1, exchange protein directly activated by cAMP 1; FGF2, fibroblast growth factor 2; FPASL, FPASL long non-coding RNA; HTS, hypertrophic scar; lncRNA, long non-coding RNA; m6A, N6-methyladenosine; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; METTL3, methyltransferase-like 3; miRNA, microRNA;

MMP1, matrix metalloproteinase 1; MN, microneedle; MOF, metal-organic framework; mTOR, mechanistic target of rapamycin; NR2F2, nuclear receptor subfamily 2 group F member 2; PAI-1, plasminogen activator inhibitor 1; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog; RhoA, Ras homolog family member A; RNA, ribonucleic acid; Smad, small mothers against decapentaplegic; SSc, systemic sclerosis; TAK1, transforming growth factor β-activated kinase 1; TAZ, tafazzin; TGF-β, transforming growth factor-β; TUG1, taurine-upregulated gene 1; uPA, urokinase plasminogen activator; YAP, Yes-associated protein.

Table SIV. Clinical translation studies targeting epigenetic drivers of cutaneous fibrosis.

First author, year	Experimental design/model (n)	Intervention and dose	Primary efficacy endpoint	Key safety/PD metric	Duration of follow-up	Ref.
Yeo *et al*, 2017	Rabbit ear scar (8)	Single 0.3 mg 5-fluorouracil MN shot	Scar height reduced by 28% comparable with daily intra-lesional 5-fluorouracil administration	Plasma below LOD; no epidermal atrophy	28 days	(102)
Yang *et al*, 2021	Rabbit ear scar (10)	5-fluorouracil 0.4 mg + triamcinolone 0.2 mg biphasic MN	Scar height reduced 38% versus 22% reduction following monotherapies	TGF-β1 protein was downregulated 34% in dermis	28 days	(103)
Zhang *et al*, 2022	Rabbit ear scar (10)	Single application of 0.5 mg SR11302 (JUN inhibitor) MN	Scar height reduced 48% versus non-separating MNs	Tip detachment only under pH<6.5 and ROS>500 µM; no unwounded-skin injury	28 days	(104)
Chien *et al*, 2022	Rabbit ear scar (12)	Single administration of 1 mg tranilast-loaded MNs	Scar height reduced 42% versus 22% for daily gel application over 21 days	Plasma <5 ng ml^{-1}; zero-order kinetics observed for 14 days	42 days	(105)
Chen *et al*, 2023	Rabbit ear scar (10)	Porphyrin-MOFs + 660 nm illumination, 10-min exposure	Scar height reduced 46% versus dark control	VEGFA was downregulated by 52%; α-SMA was downregulated by 38%	28 days	(106)
Chen *et*	Rabbit ear scar	5-aminolevulinic acid-	Scar height reduced 52%; no	Ferroptosis in fibroblasts;	56 days	(107)

al, 2025	(9)	loaded MOF-MNs + endogenous ROS	rebound at week 8	keratinocytes spared		
Yuan *et al*, 2021	Rabbit ear scar (8)	miR-29a-MSC-exo, $1x10^9$ per MN	Scar height reduced 35% versus control MN	p-Smad3 was downregulated by 44%; 80% of exosomes were released within 48 h	28 days	(108)
Zhen *et al*, 2025	Pig HTSs (6)	miR-200s-EpiSC-exo + α-TGF-β antibodies	1.2 mm flattening versus 0.6 mm after treatment with exo-only MNs	Collagen-I mRNA was downregulated by 50% at week 12	84 days	(72)

MN, microneedle; LOD, limit of detection; TGF-β1, transforming growth factor-β1; ROS, reactive oxygen species; VEGFA, vascular endothelial growth factor A; α-SMA, α-smooth muscle actin; MSC, mesenchymal stem cell; EpiSC, epidermal stem cell; exo, exosome; MOF, metal-organic framework; PDT, photodynamic therapy; HTS, hypertrophic scar.