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Transcriptional Profiling of Rapamycin-Treated Fibroblasts From Hypertrophic and Keloid Scars

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Abstract

Excess scar formation after cutaneous injury can result in hypertrophic scar (HTS) or keloid formation. Modern strategies to treat pathologic scarring represent nontargeted approaches that produce suboptimal results. Mammalian target of rapamycin (mTOR), a central mediator of inflammation, has been proposed as a novel target to block fibroproliferation. To examine its mechanism of action, we performed genomewide microarray on human fibroblasts (from normal skin, HTS, and keloid scars) treated with the mTOR inhibitor, rapamycin. Hypertrophic scar and keloid fibroblasts demonstrated overexpression of collagen I and III that was effectively abrogated with rapamycin. Blockade of mTOR specifically impaired fibroblast expression of the collagen biosynthesis genes *PLOD*, *PCOLCE*, and *P4HA*, targets significantly overexpressed in HTS and keloid scars. These data suggest that pathologic scarring can be abrogated via modulation of mTOR pathways in procollagen and collagen processing.

Keywords

microarray; hypertrophic scar; keloid; collagen; fibroblast

Hypertrophic scars (HTSs) and keloids are the result of aberrant wound healing and can cause considerable disability and disfigurement after injury. Causative factors are poorly defined and include inflammation, mechanical force, hormones, and genetics.^{2–4} Although modern treatment regimens include topical ointments, silicone sheeting, systemic and intralesional corticosteroids, laser therapy, radiation, and/or surgical extirpation, clinical outcomes remain suboptimal.⁵ This may be caused by the use of nontargeted therapies to treat pathogenic processes that are only partly understood on a molecular level.

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Clinicians have long recognized that HTS and keloids have unique clinical presentations and are distinguished by whether there is growth beyond the original wound margins.¹ However, it remains controversial as to whether these diseases represent different stages along the same spectrum of dysfunctional wound healing or whether they are distinct entities that produce excess fibrosis.⁶ Clinically, HTSs form within months after injury, tend to regress over time, and commonly affect extensor surfaces and other high-tension areas.^{1,3,6} On the other hand, keloids are more common in patients with darker pigmented skin, are more likely to recur after treatment, and may be subject to genetic and hormonal influences.⁷ Histologically, HTSs demonstrate fine collagen bundles parallel to the epidermis, myofibroblast-containing nodules, and low levels of proliferation markers. In contrast, keloids contain thick collagen bundles, lack collagen nodules, and exhibit high levels of proliferation and metabolic activity.⁸

More recently, researchers have begun to elucidate the molecular pathways driving excess scar formation. One target of particular interest is mTOR, a multifaceted regulator of cell growth and survival that has been linked to inflammatory and fibrotic processes.⁹⁻¹³ Rapamycin, a potent inhibitor of mTOR, has been shown to blunt matrix production and proliferation in fibroblasts,^{11,13} highlighting its clinical potential for the treatment of fibroproliferative scarring. However, its specific mechanism of action remains unclear, and its role in treating different types of abnormal scarring has not been previously investigated. In this study, we apply genomewide microarray analysis to examine the transcriptional profiles of human fibroblasts from normal skin, HTS, and keloid specimens after mTOR inhibition via rapamycin therapy.

MATERIALS AND METHODS

Skin Specimen Harvest

All procedures were performed in accordance with the Oregon Health and Science University institutional review board guidelines. Specimens were collected under sterile conditions in the operating room and were immediately stored in liquid nitrogen or transferred on ice for cell culture experiments. Samples were identified as keloids or HTS by expert surgeons at the time of operation and were confirmed via pathologic analysis. Patient demographics are listed in Tables 1 to 3.

Dermal Fibroblast Culture

Fibroblasts were harvested from freshly excised specimens and were maintained in low glucose DMEM (Gibco; Life Technologies Corp, Grand Island, NY) containing 20% fetal bovine serum (Gibco) as previously described.¹⁴ Cells from passages 2 or 3 were used for all experiments.

Rapamycin Treatment

Fibroblasts were washed with serum-free DMEM and were serum starved for 24 hours. Rapamycin (Ready Made Solution; Sigma Life Science, St Louis, MO) was added to fibroblast cultures at 0.02 µg/mL, and cells were collected at day 3 after treatment.

Quantitative Polymerase Chain Reaction

Whole tissue messenger RNA was isolated using the Qiagen MiniPrep Kit (Qiagen, Valencia, CA) and was reverse transcribed (High-Capacity RNA to cDNA Kit; Applied Biosystems, Foster City, CA) according to manufacturer's instructions. Complementary DNA was diluted 100 times before loading onto PCR plates. StepOne Real Time PCR system (Applied Biosystems) was used for quantitative PCR. Comparative CT (CT) method was used for quantitation. Primers and probes were purchased Taqman Gene Expression Assay (Applied Biosystem): collagen I (Hs01076751_g1), collagen III (Hs00943809_m1), PLOD1(Hs00609368_m1), PLOD2(Hs00168688_m1), PLOD3 (Hs00153670_m1), PCOLCE1(Hs00170179_m1), PCOLCE2 (Hs00203477_m1), P4HA1(Hs00914594_m1), P4HA2(Hs00990001_m1), and B2M (Hs00984230_m1).

Microarray Analysis

RNA was extracted from cultured cells as described previously. Samples were processed by the Oregon Health and Science University microarray core facility using Illumina BeadArray systems (Illumina, Inc, San Diego, CA). Raw microarray data (sample intensity files) were processed, and principal components analysis was performed using GeneSifter (PerkinElmer, Waltham, Mass); significance was evaluated using the R software environment for statistical computing (GNU project). Multiple hypothesis testing correction was performed using Benjamini-Hochberg with a false discovery threshold of 0.05. Gene set enrichment analysis was applied to detect nonrandom distributions of gene subsets. Hierarchical clustering was performed in MATLAB (Mathworks, Natick, MA), and pathway networks were constructed using Ingenuity Pathways Analysis (Ingenuity Systems, Redwood City, Calif).

Statistical Analyses

Student *t* test was used for comparing PCR values across the samples. Fibroblast gene expression experiments were performed in triplicate using a total sample size of 3 for each group. *P* values less than 0.05 were considered statistically significant.

RESULTS

Validation of Pathologic Scar Specimens

Scar types were initially determined by expert surgeons in the operating room after specimen excision and subsequently confirmed by histopathologic analysis when available. Both HTS and keloids exhibited greater levels of collagen I and III gene expression compared with normal skin. In HTS, expression of collagen I (range, 14.69–72.54 vs 0.84–3.89 relative expression; *P* < 0.05) and collagen III (range, 15.33–46.71 vs 0.92–2.86 relative expression; *P* < 0.05) was significantly greater compared with unwounded skin. Similarly, in keloids, expression of collagen I (range, 17.93–39.13 vs 0.84–3.89 relative expression; *P* < 0.05) and collagen III (range, 15.22–44.01 vs 0.92–2.86 relative expression; *P* < 0.05) was significantly increased compared with unwounded skin samples. Previous studies have shown that both HTS and keloid specimens exhibit increased levels of collagen expression, consistent with histomorphological and molecular studies demonstrating overactive collagen

matrix pathways in pathologic scars.¹⁵⁻¹⁷ These data demonstrate that HTS and keloids maintain high levels of collagen expression, suggesting that fibrogenic activity is sustained even during late scar remodeling.

Fibroblasts From HTS and Keloid Specimens are Transcriptionally More Similar Compared With Fibroblasts From Unwounded Skin

To investigate global transcriptional profiles of pathologic fibroblasts, we performed microarray studies on fibroblasts harvested from normal skin, HTS, and keloids. Clustering analysis indicated that cellular programs related to cancer, cellular movement and proliferation, connective tissue development, and cell death were significantly up-regulated ($P < 0.001$) in both HTS and keloid fibroblasts compared with normal fibroblasts from unwounded skin (Fig. 1). Many of these same categories were also down-regulated ($P < 0.001$) in both HTS and keloid fibroblasts (cancer, reproductive system disease, tissue development, inflammatory response, and connective tissue disorder). Based on the heat map analyses (Fig. 1), fibroblasts from HTS and keloids appeared to be more transcriptionally similar compared with fibroblasts from unwounded skin, suggesting some degree of transcriptional memory that is retained in cells cultured from pathologic scars.

When the transcriptional programs of HTS fibroblasts were directly compared with keloid fibroblasts, the top canonical pathways that were differentially regulated included C21-steroid hormone metabolism (eg, progestins and corticoids), immune cell cytokines, eicosanoid signaling, and arachidonic acid metabolism. Of the top 50 genes that were significantly different, 20 were down-regulated, and 30 were up-regulated in keloid compared with HTS fibroblasts (Supplementary Data Content Table 1, at <http://links.lww.com/SAP/A60>). This subanalysis suggests that HTS and keloid fibroblasts are transcriptionally distinct, consistent with the hypothesis that these forms of pathologic scarring are driven by unique molecular programs.

Rapamycin Treatment Effectively Decreases Fibroblast Expression of Collagen I and III

To test the antifibrotic potential of rapamycin, we exposed the different fibroblast populations to rapamycin in vitro. In normal fibroblasts, collagen III expression was significantly reduced after rapamycin treatment (1.0 ± 0.5 vs 0.57 ± 0.05 fold expression, $P < 0.01$), whereas collagen I expression was unchanged (1.0 ± 0.1 vs 0.86 ± 0.07 fold expression, $P = 0.18$). In HTS fibroblasts, collagen I and III expressions were significantly decreased with rapamycin exposure (1.0 ± 0.1 vs 0.69 ± 0.03 fold expression, $P < 0.01$, and 1.0 ± 0.1 vs 0.44 ± 0.01 fold expression, $P < 0.001$, respectively). In keloid fibroblasts, collagen I and III expressions were also significantly attenuated after treatment with rapamycin (1.0 ± 0.1 vs 0.63 ± 0.06 fold expression, $P < 0.01$, and 1.0 ± 0.1 vs 0.47 ± 0.03 fold expression, $P < 0.01$, respectively). These results confirm the antifibrotic effects of rapamycin on pathologic fibroblasts, substantiating its potential as a therapy for scar prevention and treatment.

Rapamycin Inhibits Collagen Expression in Fibroblasts Through Distinct Transcriptional Pathways

To further explore the mechanisms underlying rapamycin-mediated blockade of collagen expression, we performed microarray analysis on rapamycin-treated fibroblasts. From a broad perspective, rapamycin did not appear to significantly alter major transcriptional programs in any of the groups based on clustering and heat map analysis (Figs. 2–4). On closer examination, however, a set of collagen biosynthesis genes (*PLOD*, *PCOLCE*, and *P4HA*) involved in posttranslational processing of collagen was significantly down-regulated in all 3 fibroblast groups (Figs. 2A, 3A, 4A). Using quantitative polymerase chain reaction (qPCR) validation, we confirmed that rapamycin treatment significantly reduced the expression of *PLOD2* by 54%, 64%, and 46%; *PCOLCE2* by 58%, 58%, and 69%; and *P4HA2* by 60%, 74%, and 70% in normal, HTS, and keloid fibroblasts, respectively. These data suggest that rapamycin, which is thought to modulate diverse biologic processes, may have more targeted effects in human fibroblasts in vitro, specifically inhibition of collagen biosynthesis pathways.

When gene network maps were constructed based on genes significantly regulated by rapamycin, unique mechanistic pathways were identified within each fibroblast group. For normal fibroblasts, rapamycin treatment was linked to modulation of mTOR, wnt, the transcription factors nuclear factor κ B (NF κ B) and activator protein 1, and the cytokines platelet-derived growth factor (PDGF), TGF β , interleukin (IL) 1, and IL-8 (Fig. 2B). In HTS fibroblasts, rapamycin significantly regulated intracellular pathways linked to ERK, Akt, NF κ B and inflammatory targets (eg, PDGF, IL-1, IL-8, TGF β), and estrogen signaling (Fig. 3B). In keloid fibroblasts, rapamycin treatment mediated networks associated with the cytokines vascular endothelial growth factor and PDGF, the cytoskeletal proteins catenin and rac1, and estrogen signaling (Fig. 4B). Taken together, these data suggest that rapamycin modulates distinct yet overlapping mechanistic pathways in normal, HTS, and keloid fibroblasts to disrupt collagen remodeling during scar formation.

Collagen Biosynthesis Is Potentially Up-regulated in HTS and Keloid Scars Via Fibroblast mTOR

Although the mechanisms underlying excessive collagen production in vivo remain unclear, our microarray and qPCR data suggest that overactive collagen biosynthesis pathways may play an important role. To validate that these targets may be clinically relevant, we examined tissue-level gene expression of *PLOD1*, *PLOD2*, *PCOLCE*, *P4HA1*, and *P4HA2* in unwounded skin, HTS, and keloid scars. Consistent with our fibroblast studies, qPCR analysis demonstrated that the transcriptional activity of collagen processing genes was significantly up-regulated in both HTS and keloidal scar tissue compared with unwounded skin (Fig. 5). These findings indicate that posttranslational collagen remodeling programs driven by pathologic fibroblasts may be a primary characteristic of excess scarring. Furthermore, small-molecule mTOR antagonists may act specifically on these pathways to reverse dysfunctional collagen remodeling.

DISCUSSION

Microarray analyses have previously been used to study the molecular pathophysiology underlying excess scar formation. Early postburn HTS specimens demonstrated significant up-regulation of genes related to cancer, apoptosis, immune system, cytoskeletal elements, and metabolism.¹⁸ Another microarray-based study of postburn HTS identified elevated levels of genes related to ECM pathways: germline oligomeric matrix protein, matrix metalloproteinase (MMP) 16, collagen type 1 α , pleiotrophin, and thrombospondin-4.¹⁹ A third study found that HTS exhibited elevated expression of collagen, growth factor, and MMP pathways compared with normal scars.²⁰ These microarray analyses highlight the importance of transcriptional regulation of ECM pathways during HTS formation.

Microarray studies have also been applied to animal models to study wound fibroproliferation. In a pig model of dermatome-induced scarring, several biologic pathways were implicated at wounds at 20 weeks after injury.²¹ These included TGF β signaling, ECM-receptor and focal adhesions, adipogenesis, inflammation, and angiogenesis; pathways implicated in human fibrotic diseases. In a mouse model of hypertrophic-like scarring, mechanical tension was shown to directly up-regulate the expression of inflammatory pathways, chemokine signaling, and cell-matrix interactions during early scar formation.^{14,22} In addition to tissue specimens, cultured HTS fibroblasts have also been studied using microarray tools. Using IL-6 as an inflammatory stimulus, researchers have shown that HTS fibroblasts fail to up-regulate the collagenases MMP-1 and MMP-3 compared with normal fibroblasts, suggesting that defective matrix degradation pathways also contribute to excess scar deposition.²³

Keloid specimens have been shown to exhibit up-regulated expression of ECM, growth factor, and apoptosis genes based on microarray profiling. Specifically, TGF β and nerve growth factor expressions were significantly increased in keloid specimens compared with normal skin.²⁴ Another microarray study examining keloid specimens from patients of African or white ancestry demonstrated differences in caspase (apoptosis), cytokine, and mitogen-activated protein kinase pathways,²⁵ suggesting a genetic predisposition to keloid formation.

In vitro microarray studies have demonstrated increased expression of insulin-like growth factor targets and decreased expression of wnt and IL-1 signaling in keloid fibroblasts.²⁶ Treatment of these cells with hydrocortisone up-regulated the expression of connective tissue growth factor and insulin-like growth factor-binding protein-3. Further analysis implicated epigenetic modifications in keloid fibroblasts associated with altered DNA methylation and histone acetylation.²⁷ In an electron beam radiation model, irradiation modulated ECM and proliferation pathways in keloid fibroblasts via IL-6 signaling.²⁸ Interestingly, when microarray profiling was performed on keloid tissue biopsy specimens and were compared with keloid fibroblasts, there was significant discordance in gene expression patterns, indicating that wound environmental cues (eg, ECM or mechanical force) and/or nonfibroblast cells (eg, keratinocytes) play critical roles in driving keloid pathophysiology.²⁹ Taken together, these microarray-based studies corroborate our results

demonstrating that transcriptional activation of inflammatory, ECM, and cancer-related pathways may underlie both HTS and keloid formation.

Comparison of transcriptional signatures between HTS and keloid fibroblasts revealed several interesting targets that suggest avenues for future research. We found significant down-regulation of the homeobox genes *HOXB8*, *HOXC6*, *HOXC8*, and *HOXA4* (master regulators of transcription) in keloid fibroblasts, corroborating a previous study with similar findings.²⁶ Keloid fibroblast expression of hyaluronan synthase 2 was diminished in our study, consistent with the described paucity of hyaluronan in keloid dermis.³⁰ Genes that were up-regulated in keloid fibroblasts as compared with HTS fibroblasts included various metabolic enzymes (eg, threonine synthase-like 2, aldo-keto reductase family 1, and pyruvate dehydrogenase phosphatase) and intracellular signaling genes (eg, calcium/calmodulin-dependent protein kinase, phospholipase C, signal transducer, and activator of transcription). This supports the hypothesis that keloids are hypermetabolic relative to HTS and normal fibroblasts.^{6,8} Furthermore, we found differential regulation of the apoptosis-related genes ubiquitin-specific peptidase 53 and death-associated protein kinase 2, corroborating other studies implicating altered survival pathways in the tumor-like growth of keloids.^{4,25,31} Ongoing studies are being conducted to validate these targets and identify unique transcriptional programs that may differentiate hypertrophic from keloid scarring.

Inhibition of mTOR has previously been studied as an anti-fibrotic therapy in multiple organ systems. In lung fibroblasts, rapamycin produces direct antifibrotic effects independent from its immunosuppressive action.³² Rapamycin has been shown to attenuate kidney fibrosis via effects on interstitial macrophages and myofibroblasts in mice.³³ Furthermore, mTOR pathways have been demonstrated to mediate ECM accumulation in mouse models of skin fibrosis, potentially via modulation of TGF β signaling.^{34,35} In our study, rapamycin blocked collagen expression and genes involved in the posttranslational processing of collagen in all fibroblast groups, but unique transcriptional networks appeared to define the therapeutic mechanism of action for each disease type.

In normal fibroblasts, rapamycin modulated several canonical inflammatory pathways linked to scar formation, including PDGF, TGF β , IL-1, and IL-8. Intracellular targets linked to mTOR, NF κ B, and activator protein 1 were also implicated, consistent with the known inhibitory effects of rapamycin on mTOR-mediated transcription.¹¹ In HTS fibroblasts, mTOR inhibition affected inflammatory pathways identical to those in rapamycin-treated normal fibroblasts, but estrogen signaling was identified as a potential target linked to TGF β -Smad signaling. Interestingly, estrogen pathways were also detected in rapamycin-treated keloid fibroblasts, consistent with clinical case reports demonstrating improved scar outcomes with the antiestrogen agent tamoxifen.³⁶ Finally, rapamycin-treated keloid fibroblasts exhibited markedly altered vascular endothelial growth factor signaling, a pathway previously linked to keloid fibroblast proliferation and keloid progression.³⁷⁻³⁹ Our study is the first to simultaneously perform microarray analysis on HTS and keloid fibroblasts, thus revealing potential new targets for investigation. However, future studies with larger sample sizes are needed to further dissect the molecular pathways driving HTS versus keloid formation.

Our data strongly implicated a role for rapamycin in blocking posttranslational procollagen to collagen biosynthesis pathways (PLOD, PCOLCE, and P4HA) in normal and pathologic fibroblasts. PLOD2 encodes for an enzyme that hydroxylates lysyl residues within collagen peptides, producing irreversibly cross-linked collagen fibers that are characteristic of scar accumulation. Fibroblasts from systemic sclerosis patients, which overexpress PLOD2, produce matrix with increased collagen crosslinking,⁴⁰ and lung fibroblasts exposed to TGF β exhibit increased expression of PLOD2.⁴¹ Furthermore, PLOD expression is elevated in fibroblast cultures from HTS, keloids, Dupuytren fascia, and hepatic stellate cells, suggesting that PLOD plays a major role in diverse fibrotic processes.^{41,42}

PCOLCE (also known as PCPE) encodes for a glycoprotein that enhances the activity of procollagen C proteinases, enzymes that critically cleave collagen propeptides during collagen fiber assembly. PCOLCE1 has been linked to fibroblast production of stiffer collagen matrix in vitro,⁴³ and PCOLCE knockout mice exhibit impaired matrix biomechanical properties in bone and tendon because of disrupted collagen fibril morphology.⁴⁴ PCOLCE is also thought to induce collagen synthesis in ligament, liver, and cardiac fibroblasts,⁴⁵⁻⁴⁷ highlighting its potential role in scar formation across organ systems. Interestingly, PCOLCE proteins may also regulate metalloproteinase activity, suggesting a dual role in scar formation through regulation of synthesis and degradation pathways.⁴⁸

Prolyl 4-hydroxylase (P4H) is a tetrameric protein responsible for the formation of mature triple helical collagen via conversion of prolyl residues to hydroxyproline.⁴⁹ The alpha subunit (P4HA) is the active catalytic component, and inhibitors of P4HA have been shown to reduce fibrosis in an HTS model in rabbits.⁵⁰ Corticosteroids are a mainstay of scar therapy, and the topical steroid triamcinolone decreased P4HA activity and impaired collagen synthesis in human keloid and normal fibroblasts in vitro.⁵¹ Prolyl 4-hydroxylase has also been implicated in dermal fibrosis in mouse models of systemic sclerosis potentially linked to β -catenin and wnt signaling⁵²; targets were also identified in our microarray analysis.

Gene profiling studies based on invitro experiments have several limitations that should be acknowledged. First, skin cell populations such as keratinocytes, immune cells, and stem cells likely also contribute to scar formation and were not examined in this study. In addition, the use of clinical specimens is limited by the significant variability of human tissues, including differences in patient demographics, scar age, and scar location. Future studies should aim to pair unwounded skin and/or normal scars with pathologic scars from the same patient if possible. Furthermore, gene expression analyses may not completely reflect alterations in protein activity or functional phenotypes during collagen deposition. Finally, in vitro studies fail to account for the importance of matrix environment and other biophysical cues during scar formation.

In summary, our findings suggest that HTS and keloid formation may be distinct disease entities with overlapping pathways that result in excess fibrosis after injury. Profibrotic pathways linked to inflammation, ECM remodeling, and cancer regulation are significantly up-regulated in our scar specimens, consistent with current hypotheses linking aberrant

wound healing and cancer.⁵³ Although mTOR signaling regulates numerous biologic processes, application of the mTOR inhibitor rapamycin appears to specifically block posttranslational processing of collagen in human fibroblasts. These collagen biosynthesis pathways are significantly up-regulated in both HTS and keloid scars, indicating that mTOR antagonists may have a powerful role in treating fibroproliferative disease. As the molecular pathways driving pathologic scar formation become better understood, clinical therapies should be rationally designed to target dysfunctional fibrogenic pathways while minimizing adverse effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Expression profiling of fibroblasts from normal skin, HTS, and keloids. Hierarchical clustering of 250 differentially expressed genes in fibroblasts cultured from unwounded normal skin (N), HTS tissue, and keloid scar tissue (K). Dendrogram on left, heat map in the middle, and significantly regulated gene ontology categories on the right. Rows represent individual genes, and columns represent individual samples ($n = 3$ per group). Yellow and blue indicate up-regulation and down-regulation, respectively.

FIGURE 2.

Analysis of untreated versus rapamycin-treated fibroblasts from normal skin. A, Hierarchical clustering of differentially expressed genes in normal fibroblasts (N) either treated with control drug or rapamycin (R). Dendrogram on the left, and heat map on the right. Rows represent individual genes, and columns represent individual samples (n = 3 per group). Yellow and blue indicate up-regulation and down-regulation, respectively. B, Ingenuity Pathways Analysis—constructed transcriptome network based on hierarchical clustering. Solid indicates direct; dashed, indirect interactions.

FIGURE 3.

Analysis of untreated versus rapamycin-treated fibroblasts from HTS. A, Hierarchical clustering of differentially expressed genes in HTS fibroblasts treated with control drug or rapamycin (R). Dendrogram on the left, heat map on the right. Rows represent individual genes, and columns represent individual samples (n = 3 per group). Yellow and blue indicate up-regulation and down-regulation, respectively. B, Ingenuity Pathways Analysis—constructed transcriptome network based on hierarchical clustering. Solid indicates direct; dashed, indirect interactions.

FIGURE 4. Analysis of untreated versus rapamycin-treated fibroblasts from keloid scars. A, Hierarchical clustering of differentially expressed genes in keloid fibroblasts (K) either treated with control drug or rapamycin (R). Dendrogram on the left, heat map on the right. Rows represent individual genes, and columns represent individual samples (n = 3 per group). Yellow and blue indicate up-regulation and down-regulation, respectively. B, Ingenuity Pathways Analysis–constructed transcriptome network based on hierarchical clustering. Solid indicates direct; dashed, indirect interactions.

FIGURE 5.

Quantitative PCR validation of collagen biosynthesis genes in tissue specimens. Gene expression validation of tissue samples from unwounded skin, HTS, or keloid. Experiments were performed in triplicate for each sample. Values represent mean $\pm$ 1 SD. * $P < 0.05$ relative to unwounded skin samples. See Table 1 for clinical demographics.

TABLE 1

Clinical Demographics for Human Scar Tissue Studies

Tissue Studies					
Sample Type	Patient age, y	Sex	Ethnicity	Anatomical Location	Scar Age
1—Unwounded skin	65	F	White	Breast	NA
2—Unwounded skin	43	F	White	Breast	NA
3—HTS	36	F	White	Finger	>12 mo
4—HTS	60	F	White	Retroauricular	4.5 mo
5—HTS	32	F	White	Breast	7 mo
6—Keloid	41	M	White	Back	>12 mo
7—Keloid	31	F	Asian	Back	16 mo
8—Keloid	13	F	African	Earlobe	11 mo

F indicates, female; NA, not applicable; M, male.

TABLE 2

Clinical Demographics for Human Fibroblast Microarray Studies

Sample Type	Patient Age, y	Sex	Ethnicity	Anatomical Location	Scar Age
Unwounded skin	58	F	White	Breast	NA
Unwounded skin	50	F	White	Breast	NA
Unwounded skin	19	F	White	Breast	NA
HTS	36	F	White	Abdomen	15 mo
HTS	2	M	White	Cheek	2 mo
HTS	37	F	White	Chest	9 mo
Keloid	17	F	White	Hand	Unknown
Keloid	25	M	White	Neck	24 mo
Keloid	16	F	Hispanic	Earlobe	32 mo

TABLE 3

Clinical Demographics for Human Fibroblast in Vitro Studies

Sample Type	Patient Age, y	Sex	Ethnicity	Anatomical Location	Scar Age
Unwounded skin	50	F	White	Breast	NA
Unwounded skin	57	F	White	Thigh	NA
HTS	37	F	White	Chest	9 mo
HTS	39	F	White	Arms	28 mo
Keloid	25	M	White	Neck	24 mo
Keloid	17	F	White	Hand	Unknown