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Deciphering Collagen Phenotype Dynamics Regulators: Insights from In-Silico Analysis

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Abstract

Collagen (Col) types I and III are integral components in wound healing and tissue regeneration, influencing tissue development, homeostasis, and related pathologies. Col I and Col III expression changes during different stages of wound healing and understanding the regulation of collagen phenotype determination is crucial for unraveling the complexities of these processes. Transcription factors and microRNAs, directly and indirectly, play a critical role in regulating collagen expression, however, a comprehensive understanding of the factors regulating Col I and III phenotypes remains elusive. This critically analyzed published reports with focuses on various factors regulating the expression of Col I and Col III at the transcriptional and translational levels. We performed bioinformatics analysis with an input of proinflammatory mediators, growth factors, elastases, and matrix metalloproteinases and predicted transcription factors and microRNAs involved in the regulation of collagen expression. Network analysis revealed an interaction between genes, transcription factors, and microRNAs and provided a holistic view of the regulatory landscape governing collagen expression and unveils intricate interconnections. This analysis lays a founda-tional framework for guiding future research and therapeutic interventions to promote extracellular matrix remodeling, wound healing, and tissue regeneration after an injury by modulating collagen expression. In essence, this scientific groundwork offers a comprehensive exploration of the regulatory dynamics in collagen synthesis, serving as a valuable resource for advancing both basic research and clinical interventions in tissue repair.

Keywords: Bioinformatics; Collagen I; Collagen III; microRNA; Tissue regeneration; Tissue repair; Transcription factors; Wound healing;

Introduction

Collagen, the most abundant protein in the human body, constitutes approximately 25-35% of the total protein content. The term "collagen" stems from the Greek word "kolla" meaning glue, reflecting its adhesive nature in connective tissue [1]. It serves as a fibrous structural protein that imparts strength, support, and elasticity to a wide array of connective tissues. Renowned for its remarkable tensile strength, collagen is a primary building block in the construction of ligaments and tendons [2]. It serves as a crucial extracellular matrix component in various dental tissues, excluding enamel, and is prevalent in bones, cartilage, teeth, and the cornea in crystalline form [3,4].

Collagen is characterized by a distinctive structural motif wherein three parallel polypeptide strands, adopting a left-handed polyproline II-

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type (PPII) helical conformation, coil around each other in a right-handed triple helix configuration [5]. The precise arrangement involves a one-residue stagger. The densely packed PPII helices in the triple helix dictate a Gly residue at every third position, yielding a recurring XaaYaaGly sequence, where Xaa and Yaa represent any amino acid [6]. This repetitive pattern is a hallmark of all collagen types, although interruptions occur at specific locations within the triple-helical domain of nonfibrillar collagens.

Collagen assumes a critical role in tissue regeneration and wound healing [7]. After an injury, the human body initiates a complex cascade of events to repair and regenerate damaged tissues. Collagen synthesis forms an intrinsic part of this intricate process as it provides the necessary scaffolding for cell migration, proliferation, and differentiation. Moreover, collagen contributes significantly to the formation of new blood vessels, which emerge as indispensable conduits for supplying oxygen and essential nutrients to the regenerating tissues [8].

The understanding of collagen type determination during wound healing holds immense importance as it enables tissue healing and regeneration. The regulation of collagen phenotype expression greatly influences tissue functionality, mechanical properties, and response to injuries. A comprehensive understanding of collagen type determination can prove invaluable in establishing targeted therapeutic interventions to promote tissue healing and regeneration and effectively address collagen-related disorders.

Collagen I and III: tissue development, regeneration, and healing

Collagen type I and type III are fundamental components of the extracellular matrix (ECM) in various tissues, each playing distinct roles across different developmental stages [9]. Their dynamic interplay contributes significantly to tissue development, maintenance, and repair. In tissues such as skin, tendons, and bone, collagen type I is the predominant fibrillar collagen, providing tensile strength and structural integrity [10]. During the early stages of tissue development, the expression of collagen type I is essential for the formation of a robust ECM framework. Fibroblasts and osteoblasts synthesize collagen type I, creating a scaffold that sup-ports tissue architecture [11]. In the context of bone development, collagen type I fibers serve as the fundamental framework for the mineralization process, thereby endowing the skeletal structure with robust strength [12]. Concurrently, in the context of tendons, collagen type I fibers play a pivotal role in conferring mechanical durability and strength to these connective tissues [13]. Collagen III represents fibrillar collagen consistently co-localized with type I collagen [14]. Dis-tinguished by thinner fibrils compared to type I collagen, collagen III exhibits notable prevalence in tissues characterized by substantial elastic fiber content, including skin and blood vessels [15]. During the early stages of tissue development, collagen type III plays a role in the formation of the provisional matrix [16].

During tissue repair and regeneration, collagen types I and III play integral roles in the wound healing process. In the early inflammatory phase, collagen III is synthesized to form a provisional matrix that aids in cell migration and immune cell infiltration. As the wound progresses to the proliferative phase, collagen type I becomes more prominent, providing a more stable and durable matrix. The remodeling phase entails the continued synthesis and organization of collagen type I, contributing to the restoration of tissue strength [17].

Collagen Biosynthesis

Fibroblasts predominantly undertake collagen synthesis, while keratinocytes, smooth muscle cells, and vascular endothelial cells also contribute to collagen production, each displaying tissue specificity. The regulation of collagen transcriptional activities is primarily dictated by cell type, yet it can be modulated by various growth factors and cytokines [18,19]. In normal physiological conditions, collagen gene expression remains low and constant. However, during wound healing, collagen transcription rates escalate to instigate tissue repair [20]. Timely and appropriate return to basal rates is crucial; failure to do so may lead to excessive collagen production, resulting in aberrant scar formation and fibrosis. Collagen synthesis involves the transcription of genes within the nucleus, ultimately leading to the generation of heterotrimeric structures and collagen fibrils (Figure 1). The transcriptional and translational processes and post translational modification during collagen synthesis are regulated by various factors, however, the factors differentially regulating col I and col III synthesis during various phases are not clearly understood.

Regulation of collagen expression

A complex web of factors and mechanisms is involved in the tight regulation at the transcriptional and translational level. The initiation and rate of collagen gene transcription are influenced by the interaction between specific regulatory elements within the gene promoter region and transcription factors [21,22]. This dynamic regulation enables precise control of collagen synthesis in response to physiological demands and external stimuli. Several transcription factors regulate the expression of collagen type 1 (COL1A1), either directly or indirectly, by either upregulating or downregulating it. These include Sp transcription factors (Sp1/Sp3) [23], Yin Yang 1 (YY1) [24], Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) [25], Sma and Mad-related protein 2/3 complex (Smad2/3 complex) [26], Nuclear Receptor4A1 (NR4A1) [27], Activating enhancer binding Protein 2 (AP2) [28], CCAAT-binding transcription factor (CBF) [29] Friend leukemia integration 1 transcription factor (FLI1) /ERGB [30] and Krüppel-like factor 6 (KLF6)

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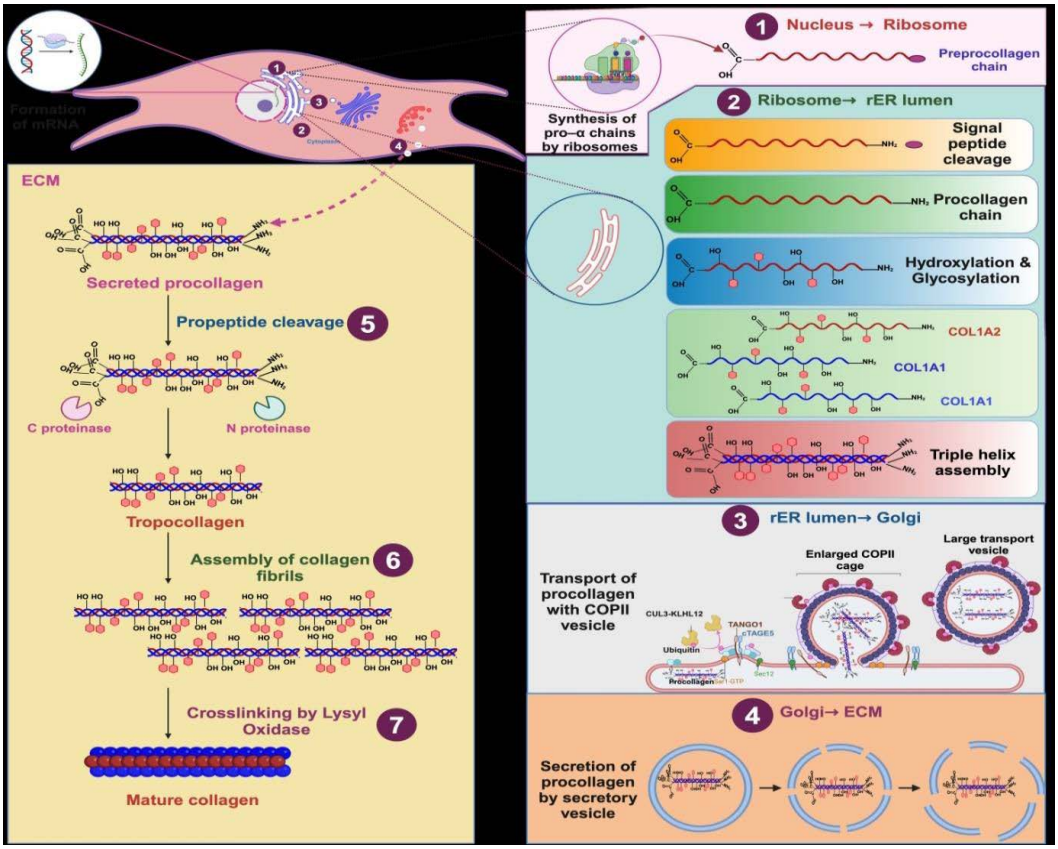

Figure 1: Collagen synthesis: Collagen synthesis starts with the synthesis of pro-α chains followed by triple helix formation (steps 1 and 2). This is followed by the transportation of the triple helix complex to the Endoplasmic reticulum and Golgi body for post-translational modification (steps 3 and 4). Procollagen is secreted outside the cell where the pro-peptide chains are cleaved and tropocollagen is formed. These tropocollagen molecules gather to form collagen fibrils, via covalent cross-linking and multiple collagen fibrils form collagen fibers (steps 5, 6, and 7).

[31], c-Myb [32], Neurogenic locus notch homolog protein 2 (NOTCH2) [33], Hypoxia Inducible Factor 1 Subunit Alpha (HIF1α) [34], Tumor necrosis factor alpha (TNF-α) [35], Yes-associated proteins (YAP)/Tafazzin (TAZ) proteins [36], Human c-Krox (hc-Krox) [37] signal transducer and activator of transcription (STAT3) [38] and Zinc finger E-box-binding homeobox 1 (ZEB1) [39].

Collagen III synthesis is also regulated by transcription factor and Smad3 plays a critical role [40]. Smad3 interacts with specific regions of the collagen 3 promoter, promoting its transcription. Collaborating with other factors, such as Smad2, AP-1, and Sp1, Smad3 helps in the regulation of collagen 3 production by forming complexes and influencing its activity. Scx regulates COL3A1 expression along with COL1A1 [41]. Post-transcriptional regulation can take place through mRNA stabilization, potentially enhancing protein synthesis, or by inducing mRNA degradation, such as through miRNAs or long non-coding RNAs (lncRNAs), likely diminishing protein synthesis. Various miRNA types play a role in regulating COL1A1 and COL3A1 expression. These include mir-21 [42], mir-200b [43], mir-196a [44], mir-10a and mir-181-c [45], mir-124 [46], mir-23b [47], mir-513b-5p [48], mir-29b [49], mir-29a [50], mir-1908 [51], mir-155 [52], mir-625-5p [53], mir-92a-1-5p [54], mir-129-5p [55], mir-133b [56], mir-98 [57], mir-21-3p [58] and mir-149 [59].

MiRNAs can also control collagen expression by regulating various enzymes involved in different stages of collagen synthesis. The miRNAs that regulates prolyl-4 hydroxylase enzymes are miR-124-3p [60], miR-155 [61], mir-17/20 [62], mir-429 [63] and miR-210 [64]. Lysyl hydroxylases and PLOD1-3 are regulated by miR-34c [65], miR-124 [66], miR-26 [67], and miR-663a [68]. The enzyme lysyl oxidase, responsible for the crosslinking oxidative deamination of epsilon-amino groups in telopeptide domains to aldehydes on lysyl and hydroxylysine residues, is regulated by miR-27 [69], miR29b [70], miR29a [71], miR-30a [72] and miR-30b [73].

Collagen degradation encompasses various proteases, with matrix metalloproteinases (MMPs) being the predominant enzymes involved [74]. These MMPs are also regulated by various miRNAs. The major MMPs involved in the degradation of Col I and Col III are MMP-1, MMP-2, MMP-8, and MMP-9. miR-192-5p can alleviate fibrosis by inhibiting Col III, MMP-1, and MMP-8 [75]. By activating

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MMP2 and MMP9, miR-513b-5p targeted the repression of COL1A1/2 expression [48]. Reduced MMP-9 expression, induced by miR-129 and miR-335, facilitated wound healing by promoting increased collagen deposition [76]. The microRNA miR-526b regulates the MMP1 gene, which plays a role in collagen degradation during both normal physiological and pathological processes [77].

Transcription factors and miRNA associated with the regulation of collagen I/III expression

The literature review reveals an obvious involvement of transcription factors and microRNAs in the regulatory mechanisms governing collagen I and III. The investigations presented in these studies elucidate the involvement of transcription factors and microRNA in the regulation of collagen expression, particularly emphasizing Col I. Nevertheless, the available literature lacks discussion on the regulatory mechanisms governing the phenotypic transition of collagen III to collagen I during the wound healing process.

The major proinflammatory mediators, enzymes, matrix metalloproteases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs) involved in the synthesis of Col I and III are Tumor Necrosis Factor-alpha (TNF-α), Transforming growth factor-β (TGF-β), Interleukin 6 (IL-6), Interleukin-1 (IL-1), Vascular endothelial growth factor (VEGF), Platelet-derived growth factor (PDGF), Prolyl hydroxylase (P4HA1-3), Lysyl hydroxylase (PLOD1-3), Lysyl oxidase (LOX), MMP1, MMP2, MMP3, MMP8, MMP9 and MMP13, TIMP1, TIMP2, Bone Morphogenic Protein 1 (BMP1), and Heat shock protein 47 (HSP47).

During tissue injury and repair, cytokines and growth factors, released by inflammatory cells like macrophages, neutrophils, and fibroblasts, regulate collagen synthesis. TGF-β, released by platelets and macrophages at the injury site, crucially stimulates collagen production by fibroblasts, promoting their proliferation and differentiation into myofibroblasts, highly active in collagen synthesis [78]. During the inflammatory phase, IL-1β and TNF-α dampen collagen synthesis while concurrently triggering the activation of MMPs mediating collagen degradation [79]. Additionally, studies indicate that IL-6 stimulates keratinocyte proliferation and collagen synthesis in dermal fibroblasts, facilitating their differentiation into myofibroblasts, and potentially contributing to skin fibrosis in systemic sclerosis patients [80].

The PLOD genes, namely PLOD1, PLOD2, and PLOD3, are responsible for encoding an enzyme known as procollagen lysyl hydroxylase, which plays a pivotal role in regulating collagen synthesis, cross-linking, and deposition [81]. Literature suggests that targeting PLOD3 could prevent pulmonary fibrosis as it is important in collagen post-translational modifications and is regulated by Wnt/β-catenin and TGFβ1/Smad3 pathways [82]. P4Hs are enzymes located in the endoplasmic reticulum that catalyze the formation of 4-hydroxyproline in collagens and other proteins. The regulation of collagen hydroxylation can significantly affect the extracellular matrix's properties and cell behavior [83]. Following triple-helical folding, Hsp47 attaches to procollagen, providing stability, and preventing premature procollagen aggregation and ER-to-Golgi export. However, Hsp47 dissociates after the procollagen shifts to the Golgi apparatus modification [84]. Following secretion from RER to Golgi, specific procollagen proteinases, such as bone morphogenetic protein 1 (BMP1), members of the ADAMTS protease family, and meprins, cleave the N- and C-terminal propeptides [85]. LOX and lysyl oxidase-like proteins catalyze the cross-linking of collagen and elastin, making ECM proteins insoluble. Literature shows that LOX is linked to renal fibrosis and cancer. Increasing LOX expression causes collagen over cross-linking through the β-arrestin/ERK/STAT3 pathway [86]. MMPs degrade extracellular matrix components and their dysregulation can lead to cancer and tissue fibrosis. There are 24 human MMPs, classified into different types [87]. TIMPs function as endogenous, selective inhibitors of MMPs, thereby modulating the equilibrium between collagen synthesis and degradation, crucial for the maintenance of tissue structural integrity [88]. Studying these inflammatory mediators, enzymes, and proteins that regulate collagen synthesis will give an idea of how Col I and Col III are regulated.

To predict various transcription factors and micro RNAs involved in collagen regulation an in-silico analysis was performed using STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) enrichment network analysis (https://string-db.org/) and Network analyst (https://www.networkanalyst.ca/) with gene input names including *TNF-α, IL-1, TGF-β, IL-6, VEGF, PDGF, P4HA1, P4HA3, PLOD1, PLOD2, PLOD3, LOX, MMP1, MMP2, MMP3, MMP8, MMP9, MMP13, TIMP1, TIMP2, BMP1* and *HSP47* along with *COL1A1, COL1A2 and COL3A1*. The list of these genes was based on our RNA seq analysisx (https://www.ncbi.nlm.nih.gov/sra; # SUB11379495), and we included the genes involved in ECM remodeling, collagen synthesis, wound healing, and tissue regeneration.

The STRING analysis (Figure 2) with a minimum required interaction score of 0.4 revealed interactions among all the input genes with *COL1A1, COL1A2,* and *COL3A1* except *VEGF*. Notably, the analysis predicted an interaction between MMP8 and *COL1A1* but not with *COL3A1* and *COL1A2* which had an interaction with MMP1, MMP2, and MMP9. STRING analysis also revealed an interaction between inflammatory cytokines IL-6 and TNF-α, growth factor TGF-β, and other regulators of collagen remodeling including TIMP2, BMP1, PLOD1, PLOD2, and PLOD3 among others (Figure 2).

The outcomes derived from the network analysis using

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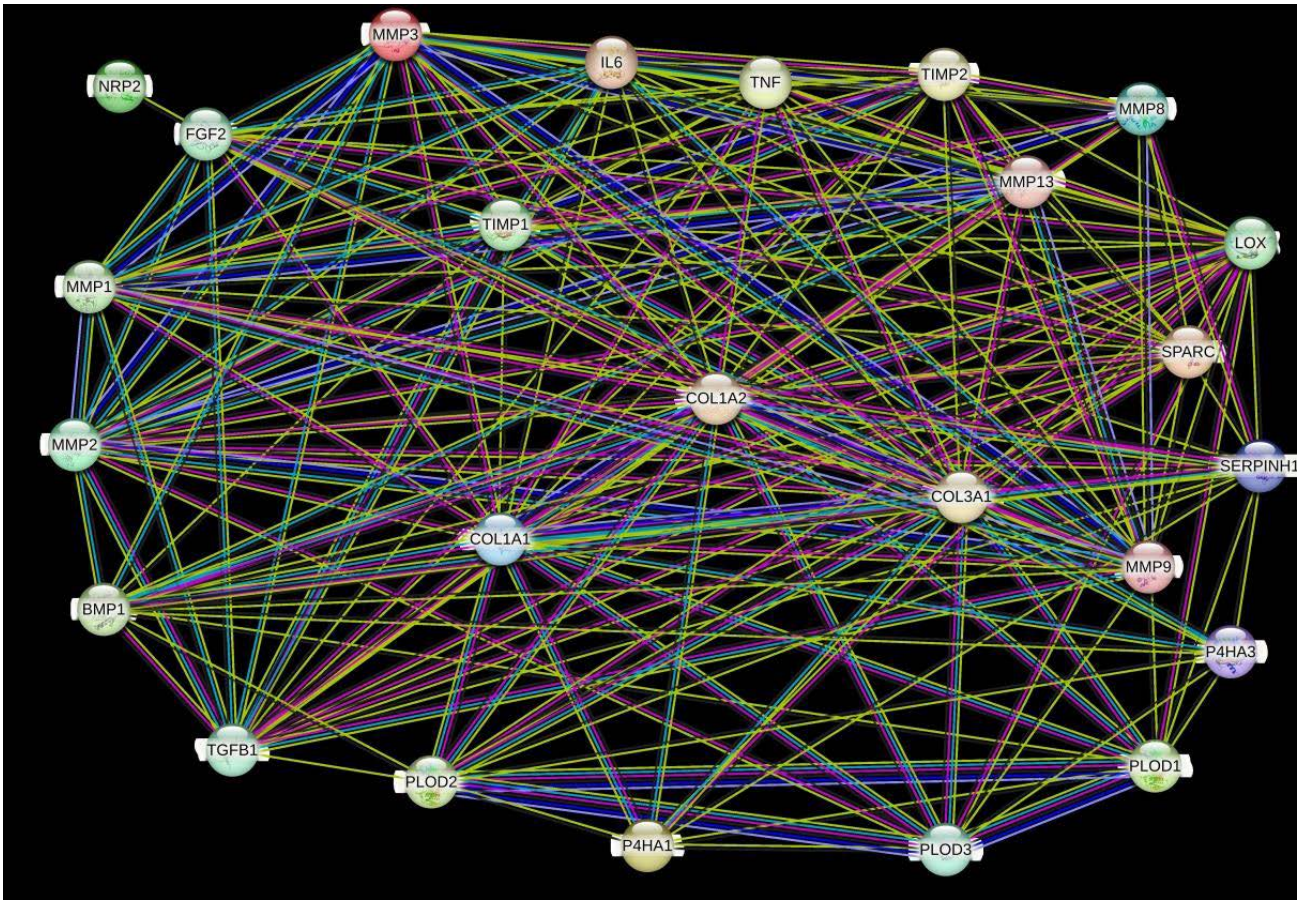

Figure 2: Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) network analysis showing the interaction between proinflammatory mediators, enzymes, and bone morphogenetic protein (BMP) 1 with collagen I and III.

the JASPAR dataset unveiled several transcription factors in association with the regulation of *COL1A1, COL1A2* and *COL 3A1* (Figure 3). Inferences drawn from the network analysis indicate that transcription factors, including, YY1, HINFP, and TP53 with a betweenness (a measure of how often a node lies on the shortest path between all pairs of nodes in a network) of 258.7, 60.15, and 45.18 respectively exercise precise regulatory control over *COL3A1* by influencing the activity of enzymes such as LOX, PLOD2, P4HA1, TIMP1, MMP3, MMP8, MMP9, and MMP13. Another transcription factor that was found to be associated with *COL3A1* was POU2F2 with a betweenness of 195.15. However, network analysis predicts that this transcription factor regulates *COL3A1* expression by regulating P4HA1 and P4HA3. P4HA3 did not show any interaction with *COL1A1* and *COL1A2*. Existing literature supports the versatile nature of the Yin Yang 1 (YY1) transcription factor, demonstrating its ability to function both as a repressor and an activator [89]. As depicted in Figure 3, YY1 exhibits the capacity to upregulate *COL3A1* through the modulation of P4HA1, LOX, and TIMP1, while simultaneously downregulating it through MMP9.

According to the NCBI gene summary, Histone H4 Transcription Factor (HINFP) encodes a transcription factor that interacts with methyl-CpG-binding protein-2 (MBD2), which forms part of the MeCP1 histone deacetylase (HDAC) complex. HINFP contributes to DNA methylation and transcriptional repression processes [90]. However, another study showed the transcriptional activator property of HNIFP when associated with its cofactors NPAT/TRRAP in HEPG2 cells [91]. Network analysis predicts the interaction of P4HA1, PLOD2, and LOX with HINF2. TP53, also known as tumor protein p53 or p53, is a tumor suppressor gene that encodes a protein that regulates cell division and prevents cancer. Literature showed that the p53 in dermal fibroblast significantly repressed the expression of MMPs [92].

The transcription factors FOXC1, NFIC, PPARG, TFAP2A, and SREB1, which interact with COL I, may regulate the expression of PLOD3, P4HA1, P4HA2, BMP1, LOX, MMP9, TIMP1, and TIMP2 (as shown in Figure 3), with respective betweenness centrality values of 547.35, 34.9, 20.79, 36.89, and 14.67. These interactions may contribute to the regulation of collagen I synthesis. FOXC1, or forkhead box C1, is a protein encoded by the FOXC1 gene. Research indicates that FOXC1 enhances the expression of MMPs, leading to reduced collagen expression and potentially

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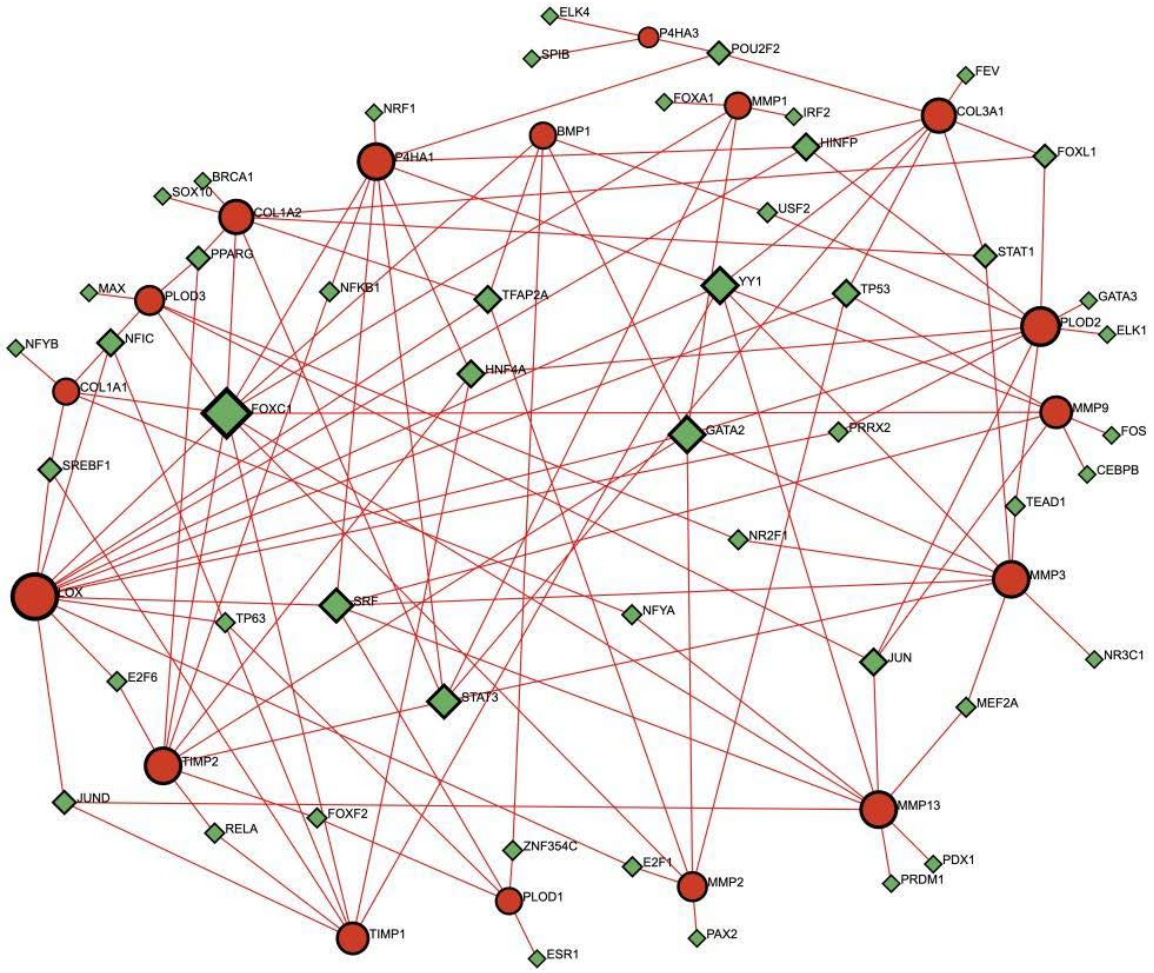

Figure 3: Network analysis for the association of input genes with transcription factors using the JASPAR dataset. Red circles (input genes) and green squares (output transcription factors).

improving myocardial repair [93]. Nuclear factor I-C (NFIC) is a member of the NFI transcription factor family, which binds to DNA via CAATT-boxes and plays roles in cellular differentiation and stem cell maintenance. In co-transfection experiments with the Colα1(I) promoter in NIH 3T3 fibroblasts, the overexpression of NF-I led to an increase in α1(I) expression [94]. PPARγ is a member of the nuclear receptor superfamily of ligand-activated transcription factors, regulating essential aspects of biology from development to metabolism. The literature revealed that the PPAR-γ agonist troglitazone modulates extracellular matrix production in human dermal fibroblasts, particularly hypertrophic scar fibroblasts (HSFBs), by acting through the PPAR-γ and Smad3, leading to the inhibition of collagen synthesis [95]. TFAP2A, also known as transcription factor AP-2 alpha, plays crucial roles in various biological processes such as development, cell proliferation, differentiation, and apoptosis. A study conducted on TFAP2A knockout fibroblasts exhibited decreased expression of α-SMA and collagen genes, indicating a critical role for TFAP2A in regulating collagen synthesis and myofibroblast differentiation [96]. Consequently, comprehensive in vitro studies investigating the regulatory dynamics of these transcription factors during the injury process hold promise in providing valuable insights into the intricate mechanisms governing collagen regulation.

The network analysis conducted using Networkanalyst. ca unveiled connections between the input genes related to collagen regulation and miRNAs regulating the expression of these genes (Figure 4). This analysis utilized the well-annotated miRNA-gene interaction database, miRTarBase v8.0). Based on the results obtained from the network analysis, it has been predicted that certain miRNAs (hsa-mir-4533, hsa-mir-5196, hsa-mir-4747-5p) have the potential to regulate the expression of alpha subunits of collagen. Additionally, the analysis also suggests that these miRNAs can influence the expression of types I and III collagens through the regulation of hsa-29b-15p, hsa-mir-29c-3p, and hsa-mir-143-3p. Other miRNAs which showed interactions between COL1A1, COL1A2 and COL3A1 with the other input genes are hsa-mir-29c-3p, hsa-mir-29b-3p, hsa-mir-143-3p, hsa-mir-335-5p, has mir-124-3p, hsa-mir-26b-5p, hsa-mir-519d-3p, hsa-mir-29a-3p, hsa-mir-363-5p, hsa-mir-6745, hsa-mir-145-5p, hsa-mir-96-5p, hsa-mir-221-3p, hsa-mir-9-5p. Many of these

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miRNAs, as reported in the literature, have been shown to inhibit collagen synthesis either by directly targeting the enzymes involved or by activating MMPs [97–102]. These findings could have significant implications for understanding the role of miRNAs in regulating the expression of collagens and could pave the way for new therapeutic interventions in the treatment of various collagen-related disorders.

Our analysis using networkanalyst.ca revealed a correlation between the selected transcription factors and miRNAs regulating expression of Col I and Col III (Figures 5 and 6). As both transcription factors and miRNAs regulate the expression of genes encoding proteins, we further analyzed the data to determine their association/co-expression.

The selected transcription factors YY1, TP53, FOXL1, and POU2F2 have a betweenness of 90547.45, 45841.9, 42337.65, and 2372.42 respectively after applying the betweenness filter as 1. According to the results of the network analysis, it was predicted that YY1 and POU2F2 may interact with various miRNA such as hsa-let-7a, hsa-mir-376b, hsa-mir-29a, etc., to directly regulate COL3A1 expression. Additionally, they may also interact with miRNA such as has-mir-515- 5p, hsa-mir-124, hsa-mir-663, hsa-mir-17, etc. to regulate the enzymes involved in collagen synthesis.

Recent literature showed that miRNA let-7a played important roles in hypoxia-related atrial fibrosis by inhibiting collagen expression and post-transcriptional repression by the JNK pathway [103]. Another study showed that miR-663a can control collagen 4 secretion in normal and ER stress conditions. Inhibiting PLOD3 expression by miR-663a reduces extracellular type IV collagen accumulation [68]. A study revealed that LINC00673 promoted breast cancer progression by acting as a ceRNA to upregulate MARK4 via miR-515-5p sponging, facilitated by YY1-mediated transcriptional activation [104].

The selected transcription factors for *COL1A1* and *COL1A2* (PPARG, TFAP2A, SREBF1, NFIC, and FOXC1) have a betweenness (interaction score between 2 genes) of 87428.3, 54703.5, 48790.9, 16903.8 and 11321 respectively. According to the network analysis, it is predicted that the selected transcription factors can interact with multiple miRNAs and regulate the expression of alpha subunits of collagen type 1. For instance, PPARG is predicted to interact with hsa-mir-563 to regulate *COL1A2*, and with hsa-mir-218 to regulate *COL1A1*. Literature also suggests that these miRNAs can regulate the subunits of collagen [105,106]. Some of the other predicted miRNAs that may regulate the expression of *COL1A1* and *COL1A2* with these transcription

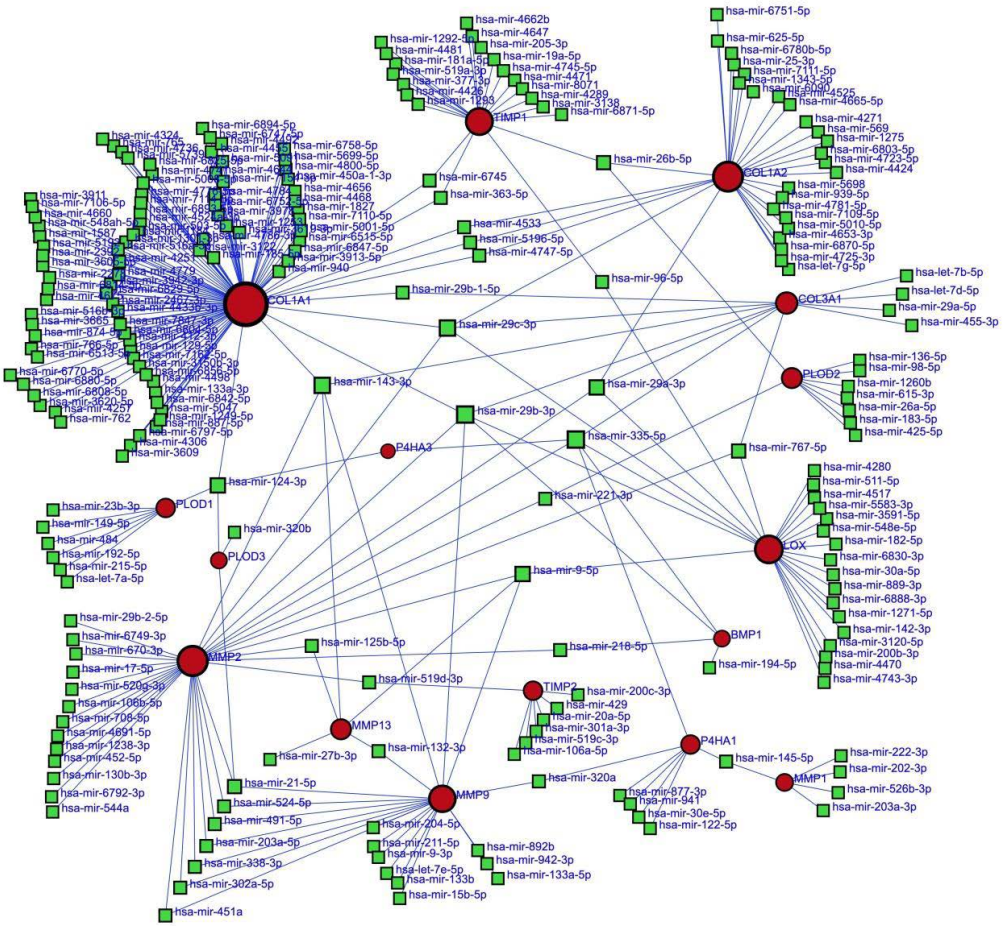

Figure 4: Network analysis for the association of input genes with microRNAs. Red circles (input genes) and green squares (output miRs).

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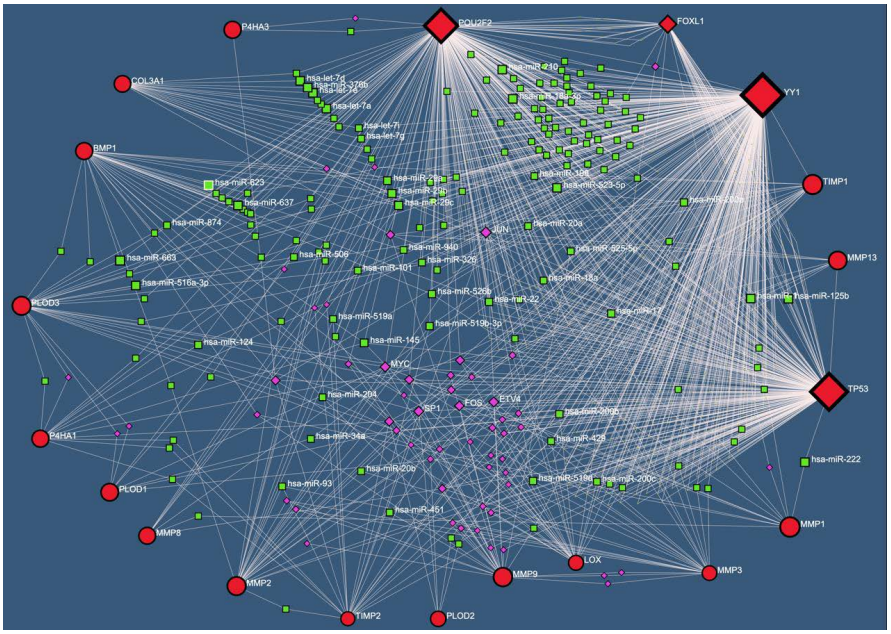

Figure 5: Network analysis of transcription factors co-expressed with microRNAs regulating COL3A1 and associated enzymes. The red circle represents input genes, and the green squares represent output microRNAs.

factors through regulating the enzymes include hsa-mir-5002-3p, hsa-mir-135-a, hsa-mir-101, hsa-mir-637, hsa-mir-339-5p, hsa-mir-21, hsa-mir-124, and has-mir-34 among others. Literature suggests that mir-637 can regulate cell proliferation and migration by suppressing MMP2 expression via the Smad3 pathway [107].

A recent study has found that overexpressing miR-101 decreased *COL1*, *COL3*, and *α-SMA* expression in Hypertrophic Scar Fibroblasts. MiR-101 mimics effectively suppressed collagen deposition in the bleomycin-induced fibrosis mouse model [108]. Another study found that inhibitors of miR-34a and miR-34c may have played a role in reducing liver fibrosis by upregulating the expression of PPARγ and downregulating the expression of α-smooth muscle actin in activated human HSCs [109]. In a recent in silico investigation that targeted the molecular mechanisms involved in both normal odontogenesis and oral/dental disorders, an interaction was found between TFAP2a and hsa-mir-135-a [110].

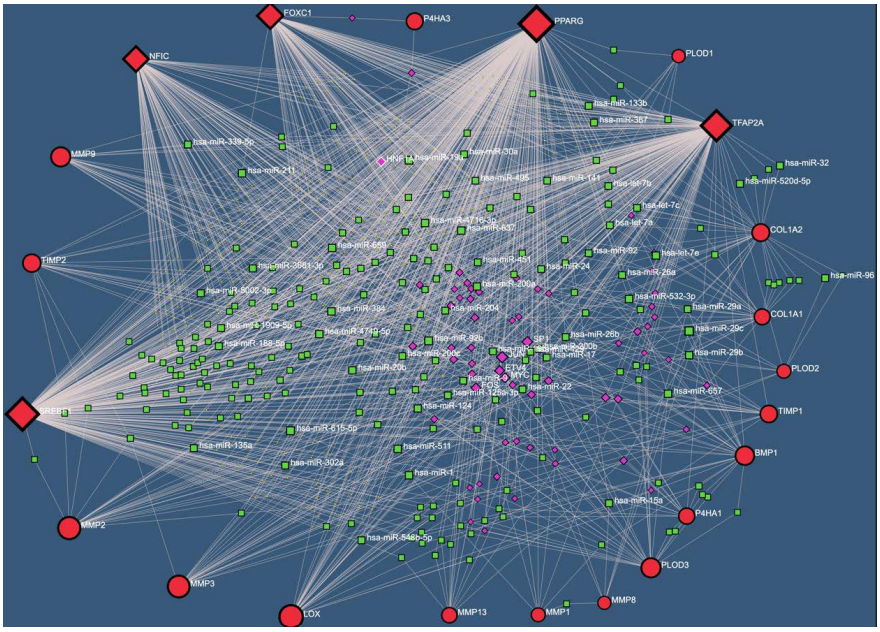

Figure 6: Network analysis of transcription factors co-expressed with microRNAs regulating COL1A1, COL1A2, and associated enzymes. The red circle represents input genes and the green squares represent output miRs.

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The outcomes derived from in-silico network analysis present a compelling proposition that transcription factors (TFs) and microRNAs (miRs) could potentially govern the expression of Col I and III. It is crucial to acknowledge that these findings are exclusively grounded in computational bioinformatics analyses. To establish the robustness and physiological relevance of these predictions, it is imperative to conduct thorough in-vitro and in-vivo validation. It is pertinent to note that in-silico analyses inherently bear limitations, and their extrapolation to biological systems necessitates empirical verification.

Conclusion

The dynamic expression of collagen types I and III throughout the distinct stages of wound healing holds pivotal significance for facilitating optimal tissue recovery without the undue formation of scar tissue, thereby contributing to the effective restoration of injured tissue function. The identification of key regulators governing the expression of Col I and III emerges as a critical pursuit in this context. Leveraging in-silico analyses, the present study offers predictions regarding several transcription factors (TFs) and microRNAs (miRNAs) with potential specificity in modulating Col I and III.

The conducted network analysis, encompassing diverse genes expressed across various stages of collagen synthesis, assumes paramount importance. This analytical approach not only elucidates the intricate interplay among these genes but also serves as a foundational framework for guiding future research endeavors and therapeutic interventions. The gleaned insights from the network analysis provide a roadmap for the design of targeted studies aimed at deciphering the regulatory mechanisms governing collagen phenotype dynamics during the different phases of wound healing. Ultimately, this scientific groundwork has the potential to inform the development of therapeutic strategies tailored to modulate collagen expression, thus influencing the outcome of wound healing and tissue regeneration.

Key Points

- The dynamic expression of collagen types I and III throughout the distinct stages of wound healing holds pivotal significance for facilitating optimal tissue recovery without scar tissue formation and thus effective restoration of injured tissue function.
- Better understanding of the regulation of collagen phenotypes is critical in unraveling the complexities of disease process utilizing the transcriptional and translational approaches.
- Leveraging in-silico analyses, the present study offers predictions regarding several transcription factors and microRNAs with potential specificity in modulating Col I and Col III.
- Network analysis revealed an interaction between genes.
- The findings lay a foundational framework for guiding future research and therapeutic in-terventions to promote extracellular matrix remodeling, wound healing, and tissue re-generation after an injury by modulating collagen expression.

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Authors' contribution:

Concept and design: RR, VR and DKA; Review of literature: RR; Drafting the article: RR and VR; Revising and editing the manuscript: VR and DKA; Final approval of the article: RR, VR and DKA

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