Review Article

Access this article online

Quick Response Code:

Website: www.carcinogenesis.com

DOI: 10.4103/jcar.JCar_24_20

Understanding the molecular mechanism associated with reversal of oral submucous fibrosis targeting hydroxylysine aldehyde-derived collagen cross-links

Smitha Sammith Shetty[1], Mohit Sharma[2], Shama Prasada Kabekkodu[3], Anil Kumar NV[4], Kapaettu Satyamoorthy[3], Raghu Radhakrishnan[5]

[1]Department of Oral Pathology, Faculty of Dentistry, Melaka Manipal Medical College, Manipal Academy of Higher Education, [2]Department of Oral Pathology, Sudha Rustagi College of Dental Sciences and Research, Faridabad, Haryana, India, [3]Department of Cell and Molecular Biology, Manipal School of Life Sciences, Manipal Academy of Higher Education, [4]Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education, [5]Department of Oral Pathology, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal, Karnataka,

Address for correspondence:
Dr. Raghu Radhakrishnan, Department of Oral Pathology, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal - 576 104, Karnataka, India.
E-mail: raghu.ar@manipal.edu

Submitted: 30-Oct-2020
Revised: 19-Feb-2021
Accepted: 24-Apr-2021
Published: 13-Aug-2021

Abstract:

Fibrosis is a pathological state characterized by excessive deposition of the extracellular matrix components leading to impaired tissue function in the affected organ. It results in scarring of the affected tissue akin to an over-healing wound as a consequence of chronic inflammation and repair in response to injury. Persistent trauma of susceptible oral mucosa due to habitual chewing of betel quid resulting in zealous healing of the mucosal tissue is one plausible explanation for the onset of oral submucous fibrosis (OSF). The irreversibility and resistance of collagen to degradation and its high potential to undergo malignant change are a major reason for morbidity in OSF. Hence, early diagnosis and timely treatment are crucial to prevent the progression of OSF to malignancy. This review focuses on the mechanistic insight into the role of collagen cross-links in advancing fibrosis and possible therapeutic targets that bring about a reversal of fibrosis. These options may be beneficial if attempted as a specific therapeutic modality in OSF as is in organ fibrosis. The upregulation of lysyl oxidase and lysyl hydroxylase has been shown to exhibit the higher levels of the hydroxylysine aldehyde-derived cross-links in fibrosis and tumor stroma promoting the tumor cell survival, resistance, and invasion. The *in silico* analysis highlights the potential drugs that may target the genes regulating collagen crosslinking.

Keywords:

Anti-fibrotic targets, collagen cross-links, fibrosis, oral submucous fibrosis, repair, treatment, wound healing

Introduction

Fibrosis is associated with a chronic inflammatory state as a result of an immune response, tissue remodeling, and repair mechanisms. The release of pro-fibrotic cytokines and growth factors results in the excessive deposition of extracellular components.[1] A similar mechanism is antecedent in oral submucous fibrosis (OSF), a chronic debilitating condition of the oral cavity, oropharynx, hypopharynx, and upper two-thirds of the esophagus, characterized by generalized submucosal fibrosis, marked rigidity, and inability to open the mouth.[2,3] Reportedly, 7%–13% of OSF has the potential to undergo malignant transformation.[4,5] The existing evidence suggests that the deregulation in collagen remodeling results in OSF.[3] Therefore, an increase in collagen synthesis in association with decreased collagen degradation is one of the plausible causes for

How to cite this article: Shetty SS, Sharma M, Kabekkodu SP, Kumar A, Satyamoorthy K, Radhakrishnan R. Understanding the molecular mechanism associated with reversal of oral submucous fibrosis targeting hydroxylysine aldehyde-derived collagen cross-links. J Carcinog 2021;20:9.

the onset of this condition. Many biological pathways and molecular mechanisms are implicated in the initiation of fibrosis.[6] A wide range of treatment modalities such as surgical therapy, drug management, and physiotherapy has been attempted with varying degrees of benefit but none can halt or reverse the fibrosis completely. The early diagnosis and sequentially early treatment of OSF are crucial to prevent its progression to malignancy.

Recent studies underscore the significance of a specific type of collagen cross-links that dictates irrevocable changes associated with a fibrotic disease.[7,8] The therapies largely target the enzymes involved in the cross-linking of collagen as it determines the stability of the extracellular matrix (ECM).[7] Understanding the pathophysiology of collagen cross-link would be important specifically target its progression and reverse fibrosis to prevent the interference of anti-fibrotic drugs in normal tissue repair, but this literature survey brings to light the potential approaches to identify the molecular targets to reverse fibrosis in OSF paving the way for evidence-based therapeutic protocols for the management of OSF.

Mechanism of Collagen Cross-Linking

Collagen is composed of three polypeptide alpha chains coiled around each other to form a triple helix configuration rich in proline and glycine. Each of the three α-chains within the molecule forms an extended left-handed helix with 18 amino acids per turn. Glycine is found at every third position of the polypeptide chain and forms the part of the repeating sequence Gly-X-Y, where X and Y can be any amino acid but are usually occupied by proline and hydroxyproline respectively.[9] Thus, all types of collagen have this basic structure forming triple helix of three polypeptide chains; however, their size, function, and tissue distribution vary considerably.[10]

An understanding of collagen cross-linking is important as it determines the physical and mechanical properties and stability of the ECM.[11] The cross-linking of the fibril-forming collagens, type I, II, III, V, XI, XXIV, and XXVII involves the posttranslational modifications of the procollagen molecules.[12] The collagen cross-linking involves two pathways, one based on Allysine, the lysine-derived aldehyde (Lys^{ald}), and the other, Hydroxyallysine, the hydroxylysine aldehyde derived (Hyl^{ald}).[11]

The initial step involves the oxidative deamination of lysine and hydroxylysine residues into aldehydes at the terminal sequence of the collagen or the telopeptide by either of the family of five lysyl oxidases (LOX and LOX-like [LOXL] 1-4), the copper metalloenzymes.[7] Further Lys^{ald} and Hyl^{ald} undergo intramolecular and intermolecular condensation with lysine, hydroxylysine, or histidine in the triple helix to form difunctional, trifunctional, or tetrafunctional cross-links.[1,11,12] In the allysine (Lys^{ald}) pathway, the Lys^{ald} in the telopeptides of the collagen molecule forms an intramolecular cross-link, aldol condensation product that matures as dehydro-histidinohydroxymerodesmosine (deH-HHMD). Lys^{ald} also undergoes intermolecular condensation to form histidinohydroxylysinonorleucine (HHL) [Figure 1a and b].[13] In the hydroxyallysine (Hyl^{ald}) pathway, the Hyl^{ald} in the telopeptide undergoes intermolecular condensation to form intermediate divalent cross-link, dehydro-hydroxylysinonorleucine and dehydro-dihydroxylysinonorleucine (deH-DHLNL), which further matures into stable pyridinoline, hydroxylysyl pyridinoline (HP), lysyl pyridinoline (LP) and pyrrole (d-PRL = deoxypyrrole; PRL = pyrrole) cross-links [Figure 1c, d and e].[13,14] In the skin, the telopeptide lysines are not hydroxylated, while in bone and cartilage, the telopeptide lysines are highly hydroxylated.[14] However, there can be a switch from Lys^{ald} to Hyl^{ald} pathways by hydroxylation of lysine at the triple helix or the telopeptide by lysyl hydroxylase enzyme (LH) and its three isoforms (LH1, LH2, and LH3) [Figure 2].[11] The hydroxylated lysine at the telopeptide has a different amino acid sequence, to that hydroxylated in the triple helix, due to different types of LH enzymes involved in hydroxylation.[9] The hydroxylation occurs only in the helical sequence Gly-X-Lys, but not when the helical sequence is Gly-Lys-Y, where lysine is in X position.[9] The Lys^{ald} pathway is noticed in the skin and cornea, whereas the

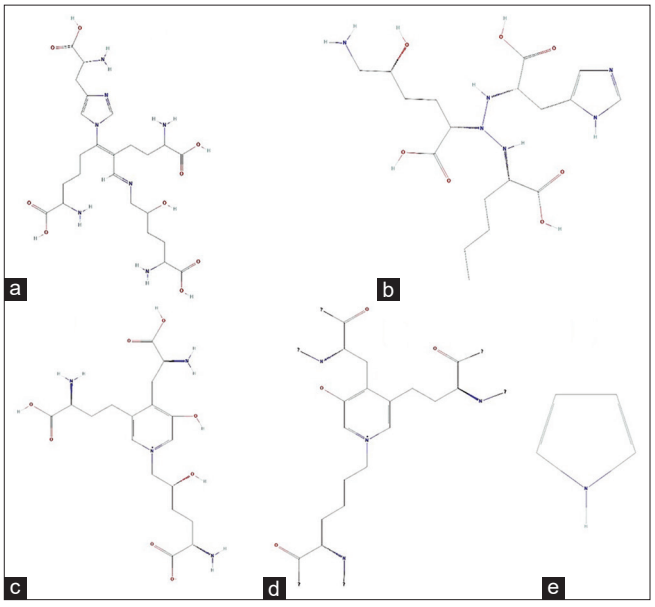

Figure 1: Mature cross-links derived from lysald and hylald pathway, (a) Dehydro-histidinohydroxymerodesmosine, (b) Histidinohydroxylysinonorleucine, (c) Hydroxylysyl pyridinoline, (d) Lysyl pyridinoline, (e) Pyrrole

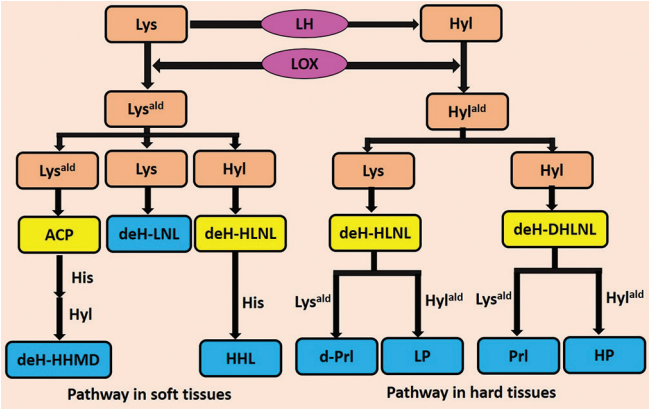

Figure 2: Schematic diagram illustrating the lys^{ald} and hyl^{ald} pathways in soft tissue and hard tissue, respectively

Hyl^{ald} pathway outweighs in bone, cartilage, tendon, dentin, and ligaments.[7,11] The Hyl^{ald} pathway usually predominates in the ECM due to the mechanical, thermal, and chemical stability of the cross-links formed.[11,15,16]

Collagen Cross-Link and Reversibility of Fibrosis

Fibrosis is the result of increased deposition of collagen accompanied by decreased proteolytic degradation of collagen, resulting due to an imbalance between the matric metalloproteinases (MMPs) and the tissue inhibitors of matrix metalloproteinases.[3] The reversibility of fibrosis may depend on the type of cross-linking of collagen as several fibrotic disorders are associated with the increased Hyl^{ald}-mediated cross-links.[17,18] The lysyl residue in the telopeptide of the collagen in the skin is not hydroxylated; hence, HHL and deH-HHMD are generally found, while the hydroxylysyl residues, the pyridinolines (HP and LP) are seen in traces.[13] In skeletal tissue, the lysyl residues are hydroxylated forming stable mature cross-links like HP, LP, and pyrrole, required for mechanical stability.[11] Reduction in collagen degradation is associated with the increased amounts of collagen cross-links derived from both enzymatic[19] and nonenzymatic (glycation) pathways.[20,21]

The accumulation of Hyl^{ald}-mediated cross-links is indicative of irreversible collagen deposition and decreased susceptibility to proteolytic enzymes. An increase of ~0.1 Schiff-base cross-links per collagen molecule results in a 2–3-fold increase in resistance to human collagenase compared with uncross-linked collagen.[19] However, it is not just the amount but also the type of cross-link that determines the digestibility of the collagen in fibrosis. Furthermore, a switch from the Lys^{ald} to the Hyl^{ald} pathway may be a significant event in soft-tissue fibrosis, rather than just the increase in the number of cross-links.[13]

LH, a member of the 2-oxoglutareate-dependent dioxygenase family[22] may have a significant role in inducing the switch in the pathway from Lys^{ald} to Hyl^{ald}.[13] Increased hydroxylation of the lysyl at the telopeptides by LH contributes to the increase in mature cross-links like HP and LP thus altering the ratio of Lys^{ald} to Hyl^{ald} cross-links.[7,12,16] Besides, various pro-fibrotic cytokines such as transforming growth factor-β (TGF-β), interleukin-4, activin A, and tumor necrosis factor-α upregulate both collagen and LH2b and bring about over hydroxylation of collagen telopeptides.[23] The Hyl^{ald}-mediated cross-links are also less susceptible to degradation by matrix metalloproteinase 1(MMP1).[1] These findings suggest that the degradability of the collagen accumulated in the fibrosis is influenced by the type of mature cross-links.

Hydroxylysine Aldehyde-Derived Cross-Links in Self-Limiting versus Progressive Fibrosis

The irreversibility of fibrosis is the result of resistance exhibited by Hyl^{ald}-mediated cross-links to proteinases, and hence, the quantity of Hyl^{ald} cross-links may be an important factor in assessing the irreversibility of fibrosis. The changes in the topology of cross-links in self-limiting and progressive forms of fibrosis may aid in strengthening these hypotheses.[7] Both the self-limiting fibrosis (wound healing) and progressive form of fibrosis initially respond with the deposition of Hyl^{ald} cross-link deH-DHLNL. However, eventually, the self-limiting form of fibrosis replaces the Hyl^{ald} cross-links with Lys^{ald} cross-links, while the hypertrophic scars retain a 1:1 ratio of both the types of cross-links and hence are resistant to degradation.[7] Further, in fibrotic conditions such as lipodermatosclerosis, there is over hydroxylation of lysine residues accompanied by increased enzymatic glycosylation of hydroxylysine residues. Glycosylation modulates the physicochemical properties of the cross-link by decreasing the susceptibility to proteolysis.[24] However, in normal skin, glycosylation occurs only to a smaller extent.[25]

Collagen Cross-Linking in Tumor Stroma

The upregulation of LOX and LOXL2 levels has been reported in numerous cancer types[2,26,27] and is known to initiate the collagen cross-linking and stiffening of the tumor stroma. LOX portents metastasis by increasing the number of collagen cross-links in the tumor stroma.[28] LOX secreted by the tumor cells facilitates the collagen crosslinking and thus stiffing of the stroma leading to the integrin-mediated formation of focal adhesions that initiate the tumor invasion.[28] LOX plays a critical role in establishing a

microenvironment that is growth-permissive and is capable of promoting metastatic invasion by enhancing tumor cell survival and persistence.[29] Similar to fibrosis, there is hydroxylation of lysine residues in tumor stroma by LH2, eventually leading to the higher levels of Hyl^{ald} cross-links and lower levels of Lys^{ald} cross-links. The change in tumor stroma promotes ECM stiffing, tumor invasion, and metastasis.[16] The tumor invasion and metastasis enhanced by LH are mediated through the upregulation of hypoxia-inducible factor-1α (HIF-1α). Both LOX and LH are upregulated in response to hypoxia and are the targets of HIF-1α; hence, hypoxia determines the type and quantity of collagen cross-links formation.[28] HIF-1α promotes tumor progression by activating the transcription of genes involved in angiogenesis, energy metabolism, and adaptive survival.[30] Therefore, the therapeutics targeting LOX and LH will abrogate not only the progression of fibrosis but also will prevent fibrosis-induced tumor invasion and metastasis.[29]

Upregulation of Lysyl Oxidases in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma

A strong correlation of activation of the TGF-β pathway in the pathogenesis of OSF has been reported suggesting the role of TGF-β as a principal regulator of fibrosis.[31] Arecoline, the principle constituent of betel quid, has been shown to upregulate the TGF-β and Thrombospondin 1 an activator of latent TGF-β in OSF.[32] While TGF-β promotes the expression of LOX both at the mRNA and protein levels in various cell lines.[33] The LOX upregulation is also facilitated with increased copper levels in OSF as LOX is copper dependent for its functional activity.[34] This suggests the excessive deposition of highly cross-linked collagen in OSF may be due to the upregulated activity of LOX [Figure 3].[2] The upregulation of LOX activity is observed in the fibroblasts cultured from OSF patients, which suggests its role in the formation of fibrotic bands in OSF.[35]

An upregulated LOX expression pattern is also seen in the invasive front of oral squamous cell carcinoma (OSCC) arising from OSF, postulating the stromal response.[2] Previous studies suggest a multifunctional role of LOX at various stages of tumorigenesis.[36] Several single nucleotide polymorphism sites have been identified in the LOX coding region such as C225G, G409C, G473A, C476A, G816A, T924G, and A1135G, where G473A shows a higher frequency of polymorphism.[33] Recently, Bhanu *et al*.[37] reported that elevated LOX in OSCC patients favored tumor growth and lymph node metastasis (LNM). The stiffened matrix due to LOX-led matrix cross-linking compressed the vasculature resulting in tissue hypoxia. The ensuing hypoxia then promoted the Rho-GTPase-dependent cytoskeletal tension leading to aberrant tumor morphogenesis, which successively augmented cellular motility resulting in metastasis. The higher expression of LOX at the invasive tumor front (ITF) resulted in a greater propensity to invade deeper structures.[37] Yu *et al*.[38] have reported a stronger LOX expression at the ITF when compared to the tumor center. Furthermore, LOX expression in basal cells of the nontumor epithelium was insignificant when compared to ITF. Saito *et al*.[39] have shown that LH2, LOX, and LOXL2 are considerably upregulated in late-stage OSCC, associated with LNM, and were allied to poor prognosis. In these tumors, augmentation of LH was particularly related to metastatic propensity as LH promoted the more stable Hyl^{ald} crosslink. Moreover, the elevation of the stromal Hyl^{ald}/Lys^{ald} cross-link ratio played a crucial role in the LNM of OSCC. This indicates that the quality of collagen crosslinking played a more significant role in cancer metastasis.[39]

Targets for Reversibility of Fibrosis

The LOX enzyme has seemingly been the main target to inhibit the covalent cross-linking of collagen as it leads to a stable, insoluble ECM due to decreased degradation by MMPs.[40] LOX inhibition affects the cross-linking of collagen in liver fibrosis and also brings about widening and splitting of fibrotic bands with disorganization and disappearance of collagen bundles over time.[40,41] Molecules targeting LOX, LOXL1, and LOXL2 have proven to be beneficial *in vivo* liver fibrosis.[40,42] The LOXL2 has been specifically targeted in phase 2 clinical trials for the treatment of myelofibrosis,[43] idiopathic pulmonary fibrosis,[44] and HIV-induced liver fibrosis[45] using the newly developed humanized monoclonal antibody, Simtuzumab.[8] Clinical trials on Simtuzumab have also been conducted for the treatment of pancreatic adenocarcinoma,[46] nonalcoholic steatohepatitis-induced fibrosis,[47] primary sclerosing cholangitis,[48] and colorectal adenocarcinoma.[49] Although Simtuzumab has shown anti-fibrotic properties in rodent fibrotic liver models by preventing and reversing fibrosis,[50] the results have not been beneficial in human trials both in the treatment of fibrosis and carcinoma possibly due to its high specificity and failure to inhibit other isoforms or the target site [Table 1].[8]

The copper-binding motif and lysyl tyrosyl quinone domain of LOX enzymes are other structural targets for its inhibition. The copper chelating agents such as D-penicillamine and tetrathiomolybdate used in *in vivo* experiments have reasonable evidence in potentially reversing fibrosis by LOX inhibition.[55,56] Clinical trials of D-penicillamine indicate its role in the reversal of

Table 1: Drug targets against lysyl oxidase tested in human clinical trials for fibrosis

Studies	LOX isoform	Drug	Class	Mechanism	Condition	Phase
Raghu *et al*., 201744]	LOXL2	Simtuzumab	Monoclonal antibody	Inhibitor	Idiopathic pulmonary fibrosis	Phase 2
Verstovsek *et al*., 2017[43]					Thrombocythaemia myelofibrosis	Phase 2
Meissner *et al*., 2016[45]					HIV-induced liver fibrosis	Phase 2
Harrison *et al*., 2018[47]					Nonalcoholic steatohepatitis-induced fibrosis	Phase 2
Muir *et al*., 201948]					Primary sclerosing cholangitis	Phase 2
Benson *et al*., 2017[46]					Pancreatic adenocarcinoma	Phase 2
Hecht *et al*., 2017[49]					Metastatic KRAS mutant colorectal adenocarcinoma	Phase 2
Bhusnurmath *et al*., 1991[51]	Copper binding motif	D-penicillamine	Copper chelating agents	Inhibitor	Childhood cirrhosis-associated fibrosis	
Pradhan *et al*., 1995[52]					Childhood cirrhosis-associated fibrosis	
Selman *et al*., 1998[53]					Idiopathic pulmonary fibrosis	
Clements *et al*., 1999[64]					Diffuse systemic sclerosis	
Keiser and Sjoerdsma, 1967[54]	LTQ domain	β-aminopropionitrile	Small molecule	Inhibitor	Scleroderma	-

LOX: Lysyl oxidase, LOXL2: Lysyl oxidase-like 2, LTQ: Lysyl tyrosyl quinone, KRAS Gene: Kirsten Rat Sarcoma Gene

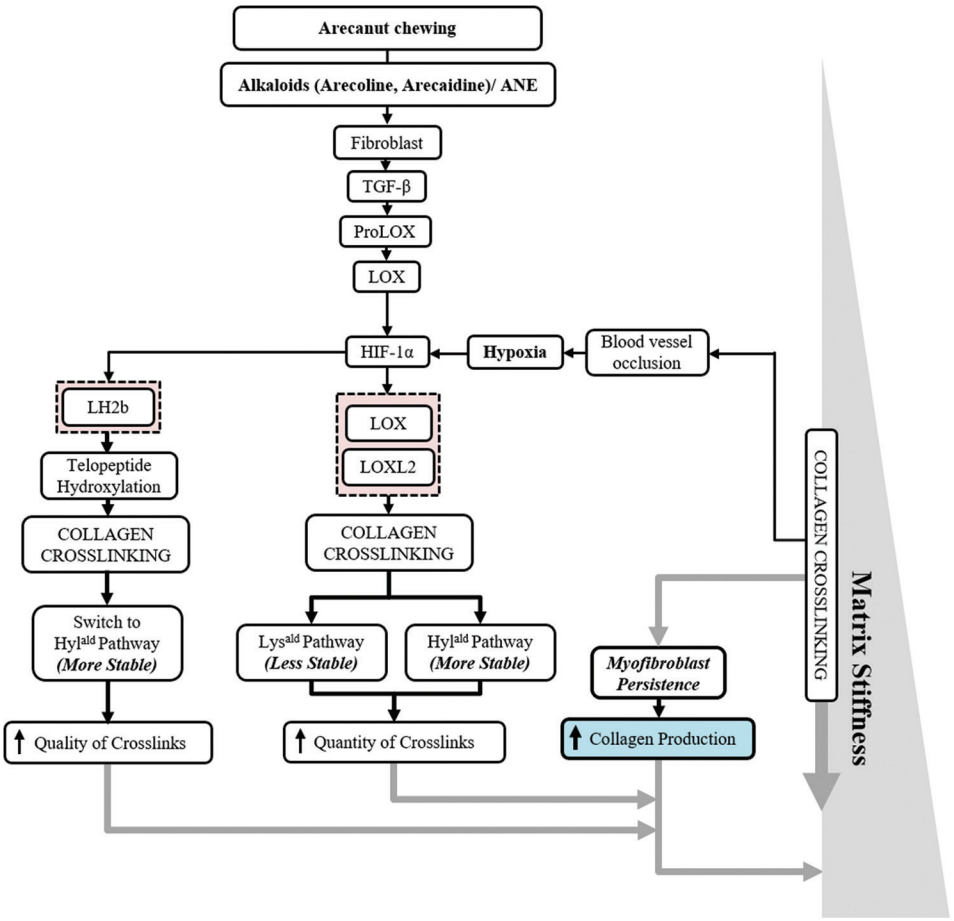

Figure 3: Schematic diagram illustrating the mechanism of collagen crosslinking in oral submucous fibrosis

childhood cirrhosis bringing about a significant decrease in fibrosis.[51,52] However, D-penicillamine failed to prevent the progressive course of Prednisone-treated idiopathic pulmonary fibrosis.[53] β-aminopropionitrile (BAPN) is another extensively studied LQT domain inhibitor of LOX, which forms an irreversible covalent complex.[57] BAPN has been widely studied *in vivo* to reverse liver, peritoneal, and myocardial fibrosis.[58,59] Clinical trials of BAPN have been conducted on scleroderma patients with limited therapeutic effects.[54] The other important target known to attenuate irreversible fibrosis is the LH enzyme, which is crucial for the formation of the stable

Table 2: Potential drug interactions using the drug-gene interaction database

Gene	Drug	Interaction type	Sources
HIF1A	Carvedilol	Modulator	DrugBank: https://www.dgidb.org/genes/HIF1A#_interactions
HIF1A	Nitroglycerin	Inhibitor	MyCancerGenomeClinicalTrial: https://www.dgidb.org/sources/MyCancerGenomeClinicalTrial
HIF1A	Chembl1080759	-	TdgClinicaltrial: https://www.dgidb.org/sources/TdgClinicalTrial
HIF1A	Deferoxamine	-	NCI: https://www.cancer.gov/
HIF1A	Geldanamycin	-	NCI: https://www.cancer.gov/
HIF1A	Noscapine	-	TdgClinicalTrial: https://www.dgidb.org/sources/TdgClinicalTrial
HIF1A	Epoetin alfa	-	NCI: https://www.cancer.gov/
HIF1A	2-methoxyestradiol	-	DrugBank: https://go.drugbank.com
HIF1A	Pimonidazole	-	NCI: https://www.cancer.gov/
HIF1A	Chembl426560	-	DrugBank: https://go.drugbank.com
HIF1A	Chembl299763	-	TdgClinicalTrial: https://www.dgidb.org/sources/TdgClinicalTrial
HIF1A	Sunitinib	-	CGI: https://www.cancergenomeinterpreter.org/home
LOXL2	Simtuzumab	Inhibitor	ChEMBL: https://www.ebi.ac.uk/chembl/
PLOD2	Ascorbate	Cofactor	DrugBank: https://go.drugbank.com

HIF1A: Hypoxia-inducible factor 1-alpha, LOXL2: Lysyl oxidase-like 2, PLOD2: Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-encoding LH2

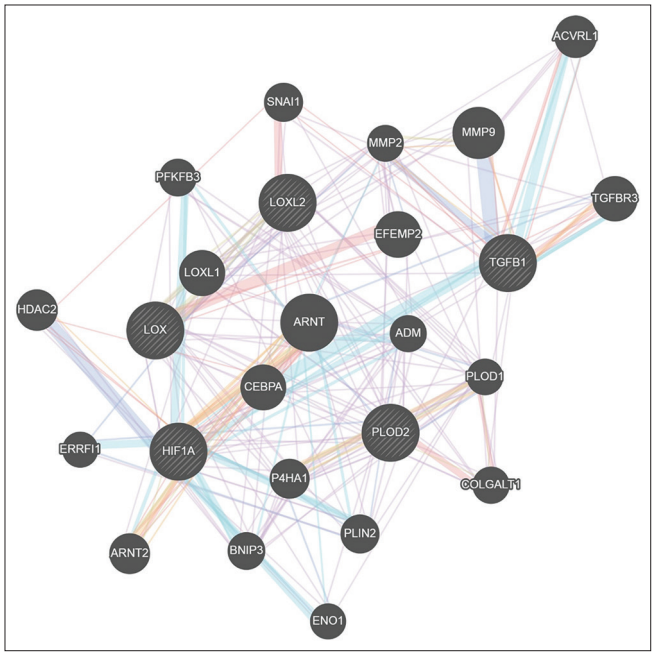

Figure 4: Construction of gene interaction network for the genes LOX, LOXL2, PLOD2, TGFB1, and HIF1A. LOX: Lysyl oxidase, LOXL2: LOX like (LOXL) 2, PLOD2: Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2- encoding LH2, TGFB1: Transforming Growth Factor Beta 1, HIF1A: Hypoxia-inducible factor 1-alpha

mature Hyl^{ald}-derived cross-links.[60,61] Minodoxil, an FDA-approved drug for alopecia and hypertension reduces LH activity by decreasing the LH1 mRNA levels and also limits the total number of hydroxylysines available for cross-link formation.[22,62] In recent years, new patents on next-generation small molecule inhibitors against LOXL2 have been released; however, the humanized clinical trials in future years will validate its potential.[63] These studies further strengthen the hypothesis that reversal of fibrosis in OSF may be possible targeting the LOX enzyme which may thereby inhibit the cross-linking of collagen.

In-Silico Analysis

In-silico analysis was performed for the genes encoding various enzymes and growth factors favoring Hyl^{ald}-mediated collagen cross-linking and fibrosis. Gene-Interaction Network was constructed for the genes *LOX, LOXL2, PLOD2* (Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2-encoding LH2) TGF-β 1, and HIF-1α using GeneMANIA Cytoscape[Figure 4]. Potential drugs targeting these genes were predicted using the drug-gene interaction database [Table 2].

Conclusion

The present review provides a mechanistic insight that irreversible fibrosis is not merely due to the abnormal accumulation of collagen accompanied by decreased collagenase degradation but is a result of the deposition of stable mature Hyl^{ald}-derived cross-links that are resistant to degradation. The upregulation of LOX and LH exhibits higher levels of Hyl^{ald}-derived cross-links in fibrosis and tumor stroma promoting the tumor cell survival, resistance, and invasion. The LOX and LH enzymes involved in collagen cross-linking are the potential targets to interfere with the fibrotic process and prevent further fibrosis-induced tumor invasion and metastasis. Recent treatment modalities for the reversal of fibrosis have focused on targeting the collagen cross-links along with decreased collagen synthesis. The nature and the type of cross-links or pathways involved in OSF are yet to be explored. A thorough understanding of the collagen cross-links in OSF will determine whether the outcome of the disease is reversible or not. Based on these findings, the decision for an appropriate therapeutic strategy based on evidence is timely and imminent for the treatment of OSF. *In-silico* analysis revealed the potential drug candidates for future research on anti-fibrotic

therapies that may target the genes regulating collagen cross-linking.

Financial support and sponsorship

This work was supported by the Science and Engineering Research Board (SERB), Department of Science and Technology, Government of India sanction order No. "EMR/2017/002792" dated September 25, 2018.

Conflicts of interest

All the authors of the manuscript hereby state that there is no financial implication or personal relationship with other people or organization that could inappropriately influence the outcome of this work.

References

1. van der Slot-Verhoeven AJ, van Dura EA, Attema J, Blauw B, Degroot J, Huizinga TW, *et al.* The type of collagen cross-link determines the reversibility of experimental skin fibrosis. Biochim Biophys Acta 2005;1740:60-7.
2. Trivedy C, Warnakulasuriya KA, Hazarey VK, Tavassoli M, Sommer P, Johnson NW. The upregulation of lysyl oxidase in oral submucous fibrosis and squamous cell carcinoma. J Oral Pathol Med 1999;28:246-51.
3. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis – A collagen metabolic disorder. J Oral Pathol Med 2005;34:321-8.
4. Phulari RG, Dave EJ. A systematic review on the mechanisms of malignant transformation of oral submucous fibrosis. Eur J Cancer Prev 2020;29:470-3.
5. Ekanayaka RP, Tilakarantne WM. Oral submucous fibrosis: Review on mechanisms of malignant transformation. Oral Surg Oral Med Oral Pathol Oral Radiol 2016;122:192-9.
6. Sharma M, Shetty SS, Radhakrishnan R. Oral submucous fibrosis as an overhealing wound: Implications in malignant transformation. Recent Pat Anticancer Drug Discov 2018;13:272-91.
7. Piersma B, Bank RA. Collagen cross-linking mediated by lysyl hydroxylase 2: An enzymatic battlefield to combat fibrosis. Essays Biochem 2019;63:377-87.
8. Chen W, Yang A, Jia J, Popov YV, Schuppan D, You H. Lysyl oxidase (LOX) family members: Rationale and their potential as therapeutic targets for liver fibrosis. Hepatology 2020;72:729-41.
9. Gjaltema RA, Bank RA. Molecular insights into prolyl and lysyl hydroxylation of fibrillar collagens in health and disease. Crit Rev Biochem Mol Biol 2017;52:74-95.
10. Gelse K, Pöschl E, Aigner T. Collagens – Structure, function, and biosynthesis. Adv Drug Deliv Rev 2003;55:1531-46.
11. Eyre DR, Paz MA, Gallop PM. Cross-linking in collagen and elastin. Annu Rev Biochem 1984;53:717-48.
12. Reiser K, McCormick RJ, Rucker RB. Enzymatic and nonenzymatic cross-linking of collagen and elastin. FASEB J 1992;6:2439-49.
13. Brinckmann J, Neess CM, Gaber Y, Sobhi H, Notbohm H, Hunzelmann N, *et al.* Different pattern of collagen cross-links in two sclerotic skin diseases: Lipodermatosclerosis and circumscribed scleroderma. J Invest Dermatol 2001;117:269-73.
14. Bailey AJ, Paul RG. The mechanisms and consequences of the maturation and ageing of collagen. Proc Indian Acad Sci 1999;111:57-69.
15. Rucker RB, Murray J. Cross-linking amino acids in collagen and elastin. Am J Clin Nutr 1978;31:1221-36.
16. Chen Y, Terajima M, Yang Y, Sun L, Ahn YH, Pankova D, *et al.* Lysyl hydroxylase 2 induces a collagen cross-link switch in tumor stroma. J Clin Invest 2015;125:1147-62.
17. Istok R, Bély M, Stancíková M, Rovenský J. Evidence for increased pyridinoline concentration in fibrotic tissues in diffuse systemic sclerosis. Clin Exp Dermatol 2001;26:545-7.
18. Ricard-Blum S, Bresson-Hadni S, Vuitton DA, Ville G, Grimaud JA. Hydroxypyridinium collagen cross-links in human liver fibrosis: Study of alveolar echinococcosis. Hepatology 1992;15:599-602.
19. Vater CA, Harris ED Jr, Siegel RC. Native cross-links in collagen fibrils induce resistance to human synovial collagenase. Biochem J 1979;181:639-45.
20. Verzijl N, DeGroot J, Thorpe SR, Bank RA, Shaw JN, Lyons TJ, *et al.* Effect of collagen turnover on the accumulation of advanced glycation end products. J Biol Chem 2000;275:39027-31.
21. Mott JD, Khalifah RG, Nagase H, Shield CF 3rd, Hudson JK, Hudson BG. Nonenzymatic glycation of type IV collagen and matrix metalloproteinase susceptibility. Kidney Int 1997;52:1302-12.
22. Shao S, Zhang X, Duan L, Fang H, Rao S, Liu W, *et al.* Lysyl hydroxylase inhibition by minoxidil blocks collagen deposition and prevents pulmonary fibrosis via TGF- β 1/Smad3 signaling pathway. Med Sci Monit 2018;24:8592-601.
23. van der Slot AJ, van Dura EA, de Wit EC, De Groot J, Huizinga TW, Bank RA, *et al.* Elevated formation of pyridinoline cross-links by profibrotic cytokines is associated with enhanced lysyl hydroxylase 2b levels. Biochim Biophys Acta 2005;1741:95-102.
24. Brinckmann J, Notbohm H, Tronnier M, Açil Y, Fietzek PP, Schmeller W, *et al.* Overhydroxylation of lysyl residues is the initial step for altered collagen cross-links and fibril architecture in fibrotic skin. J Invest Dermatol 1999;113:617-21.
25. Bailey AJ, Bazin S, Delaunay A. Changes in the nature of the collagen during development and resorption of granulation tissue. BBA Protein Struct 1973;328:383-90.
26. Gao Y, Xiao Q, Ma H, Li L, Liu J, Feng Y, *et al.* LKB1 inhibits lung cancer progression through lysyl oxidase and extracellular matrix remodeling. Proc Natl Acad Sci U S A 2010;107:18892-7.
27. Salvador F, Martin A, López-Menéndez C, Moreno-Bueno G, Santos V, Vázquez-Naharro A, *et al.* Lysyl oxidase-like protein LOXL2 promotes lung metastasis of breast cancer. Cancer Res 2017;77:5846-59.
28. Yamauchi M, Barker TH, Gibbons DL, Kurie JM. The fibrotic tumor stroma. J Clin Invest 2018;128:16-25.
29. Cox TR, Bird D, Baker AM, Barker HE, Ho MW, Lang G, *et al.* LOX-mediated collagen crosslinking is responsible for fibrosis-enhanced metastasis. Cancer Res 2013;73:1721-32.
30. Ji F, Wang Y, Qiu L, Li S, Zhu J, Liang Z, *et al.* Hypoxia inducible factor 1α-mediated LOX expression correlates with migration and invasion in epithelial ovarian cancer. Int J Oncol 2013;42:1578-88.
31. Khan I, Agarwal P, Thangjam GS, Radhesh R, Rao SG, Kondaiah P. Role of TGF-β and BMP7 in the pathogenesis of oral submucous fibrosis. Growth Factors 2011;29:119-27.
32. Khan I, Kumar N, Pant I, Narra S, Kondaiah P. Activation of TGF-β pathway by areca nut constituents: A possible cause of oral submucous fibrosis. PLoS One 2012;7: e51806.
33. Thorawat A, Nandimath K, Hiremath S, Naikmasur VG. Molecular screening of lysyl oxidase G473A polymorphism in oral submucous fibrosis. J Oral Maxillofac Pathol 2014;18:207-10.
34. Trivedy CR, Warnakulasuriya KA, Peters TJ, Senkus R, Hazarey VK, Johnson NW. Raised tissue copper levels in oral submucous fibrosis. J Oral Pathol Med 2000;29:241-8.
35. Ma RH, Tsai CC, Shieh TY. Increased lysyl oxidase activity in fibroblasts cultured from oral submucous fibrosis associated with betel nut chewing in Taiwan. J Oral Pathol Med 1995;24:407-12.
36. Shieh TM, Lin SC, Liu CJ, Chang SS, Ku TH, Chang KW. Association of expression aberrances and genetic polymorphisms of lysyl oxidase with areca-associated oral tumorigenesis. Clin Cancer Res 2007;13:4378-85.
37. Bhanu U, Natarajan S, Manaktala N, Boaz K, Joshi R, Deepak S, *et al.*

Recognition of lysyl oxidase as a potential predictive biomarker for oral squamous cell carcinoma: An immunohistochemical study. Minerva Stomatol 2020;69:360-9.
38. Yu M, Shen W, Shi X, Wang Q, Zhu L, Xu X, *et al.* Upregulated LOX and increased collagen content associated with aggressive clinicopathological features and unfavorable outcome in oral squamous cell carcinoma. J Cell Biochem 2019;120:14348-59.
39. Saito T, Uzawa K, Terajima M, Shiiba M, Amelio AL, Tanzawa H, *et al.* Aberrant collagen cross-linking in human oral squamous cell carcinoma. J Dent Res 2019;98:517-25.
40. Liu SB, Ikenaga N, Peng ZW, Sverdlov DY, Greenstein A, Smith V, *et al.* Lysyl oxidase activity contributes to collagen stabilization during liver fibrosis progression and limits spontaneous fibrosis reversal in mice. FASEB J 2016;30:1599-609.
41. Ikenaga N, Peng ZW, Vaid KA, Liu SB, Yoshida S, Sverdlov DY, *et al.* Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. Gut 2017;66:1697-708.
42. Zhao W, Yang A, Chen W, Wang P, Liu T, Cong M, *et al.* Molecular basis of disease inhibition of lysyl oxidase-like 1 (LOXL1) expression arrests liver fibrosis progression in cirrhosis by reducing elastin crosslinking. BBA Mol Basis Dis 2018;1864:1129-37.
43. Verstovsek S, Savona MR, Mesa RA, Dong H, Maltzman JD, Sharma S, *et al*. A phase 2 study of simtuzumab in patients with primary, post-polycythaemia vera or post-essential thrombocythaemia myelofibrosis. Br J Haematol 2017;176:939-49.
44. Raghu G, Brown KK, Collard HR, Cottin V, Gibson KF, Kaner RJ, *et al*. Efficacy of simtuzumab versus placebo in patients with idiopathic pulmonary fibrosis: A randomised, double-blind, controlled, phase 2 trial. Lancet Respir Med 2017;5:22-32.
45. Meissner EG, McLaughlin M, Matthews L, Gharib AM, Wood BJ, Levy E, *et al.* Simtuzumab treatment of advanced liver fibrosis in HIV and HCV-infected adults: Results of a 6-month open-label safety trial. Liver Int 2016;36:1783-92.
46. Benson AB, Wainberg ZA, Hecht JR, Vyushkov D, Dong H, Bendell J, *et al*. A Phase II randomized, double-blind, placebo-controlled study of simtuzumab or placebo in combination with gemcitabine for the first-line treatment of pancreatic adenocarcinoma. Oncologist 2017;22:241.
47. Harrison SA, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, *et al.* Simtuzumab is ineffective for patients with bridging fibrosis or compensated cirrhosis caused by nonalcoholic steatohepatitis. Gastroenterology 2018;155:1140-53.
48. Muir AJ, Levy C, Janssen HL, Montano-Loza AJ, Shiffman ML, Caldwell S, *et al.* Simtuzumab for primary sclerosing cholangitis: Phase 2 study results with insights on the natural history of the disease. Hepatology 2019;69:684-98.
49. Hecht JR, Benson AB, Vyushkov D, Yang Y, Bendell J, Verma U. A phase II, randomized, double-blind, placebo-controlled study of simtuzumab in combination with FOLFIRI for the second□line treatment of metastatic KRAS mutant colorectal adenocarcinoma. Oncologist 2017;22:243.
50. Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M, *et al.* Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. Nat Med 2010;16:1009-17.
51. Bhusnurmath SR, Walia BN, Singh S, Parkash D, Radotra BD, Nath R. Sequential histopathologic alterations in Indian childhood cirrhosis treated with d-penicillamine. Hum Pathol 1991;22:653-8.
52. Pradhan AM, Bhave SA, Joshi VV, Bavekar AR, Pandit AN, Tanner MS. Reversal of Indian Childhood cirrhosis by D-pencillamine therapy. J Pediatr Gastroenterol Nutr 1995;20:28-35.
53. Selman M, Carrillo G, Salas J, Padilla RP, Perez-Chavira R, Sansores R, *et al.* Colchicine, D-pencillamine, and prednisone in the treatment of idiopathic pulmonary fibrosis: A controlled clinical trial. Chest 1998;114:507-12.
54. Keiser HR, Sjoerdsma A. Studies on beta-aminopropionitrile in patients with scleroderma. Clin Pharmacol Ther 1967;8:593-602.
55. Brewer GJ, Ullenbruch MR, Dick R, Olivarez L, Phan SH. Tetrathiomolybdate therapy protects against bleomycin-induced pulmonary fibrosis in mice. J Lab Clin Med 2003;141:210-6.
56. Geismar LS, Hennessey S, Reiser KM, Last JA. D-penicillamine prevents collagen accumulation in lungs of rats given bleomycin. Chest 1986;89:153S-154S.
57. Hajdú I, Kardos J, Major B, Fabó G, Lőrincz Z, Cseh S, *et al.* Inhibition of the LOX enzyme family members with old and new ligands. Selectivity analysis revisited. Bioorg Med Chem Lett 2018;28:3113-8.
58. Rosin NL, Sopel MJ, Falkenham A, Lee TD, Légaré JF. Disruption of collagen homeostasis can reverse established age-related myocardial fibrosis. Am J Pathol 2015;185:631-42.
59. Harlow CR, Wu X, van Deemter M, Gardiner F, Poland C, Green R, *et al.* Targeting lysyl oxidase reduces peritoneal fibrosis. PLoS One 2017;12:e0183013.
60. Mercer DK, Nicol PF, Kimbembe C, Robins SP. Identification, expression, and tissue distribution of the three rat lysyl hydroxylase isoforms. Biochem Biophys Res Commun 2003;307:803-9.
61. van der Slot AJ, Zuurmond AM, Bardoel AF, Wijmenga C, Pruijs HE, Sillence DO, *et al*. Identification of PLOD2 as telopeptide lysyl hydroxylase, an important enzyme in fibrosis. J Biol Chem 2003;278:40967-72.
62. Hautala T, Heikkinen J, Kivirikko KI, Myllylä R. Minoxidil specifically decreases the expression of lysine hydroxylase in cultured human skin fibroblasts. Biochem J 1992;283:51-4.
63. Chopra V, Sangarappillai RM, Romero-Canelón I, Jones AM. Lysyl Oxidase Like□2 (LOXL2): An emerging oncology target. Adv Ther 2020;3:1900119.
64. Clements PJ, Furst DE, Wong WK, Mayes M, White B, Wigley F, Weisman MH, *et al.* High-dose versus low-dose D-penicillamine in early diffuse systemic sclerosis: analysis of a two-year, double-blind, randomized, controlled clinical trial. Arthritis Rheum. 1999;42:1194-203.