

Inducible Animal Models of Skin Fibrosis; Updated Review of the Literature

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Abstract

Fibrosis is a common and mostly progressive pathological outcome in various chronic inflammatory disorders. Dermal (skin) fibrosis, which is associated with intense skin lesions, is a result of an uncontrolled healing process in the dermis, particularly disproportionate fibroblast proliferation and Extracellular Matrix (ECM) production. Animal models are substantial tools in biomedical investigations and have been considerably employed to evaluate miscellaneous features of diseases that cannot be demonstrated otherwise in humans. To date, various skin fibrosis models have been generated, including the transgene and/or genetic models and chemical and drug-induced models. However, genetic models are sophisticated and need access to convoluted methods. Accordingly, the introduction of affordable and easy to generate fibrosis models in the skin is crucial. Here, we aimed to introduce the chemical/drug-induced skin fibrosis animal models to provide an updated list of available approaches.

Keywords: Fibrosis [[MeSH](#)]; Models Animal [[MeSH](#)]; Skin [[MeSH](#)]; Bleomycin [[MeSH](#)]; Hypochlorous Acid [[MeSH](#)]; Vinyl Chloride [[MeSH](#)]

Highlights

- To date, various skin fibrosis models have been generated, including the transgene and/or genetic models and chemical and drug-induced models.
- Here, we aimed to introduce the chemical/drug-induced skin fibrosis animal models to provide an updated list of available approaches.

Introduction

Fibrosis is typically defined as the runaway production, activation, and deposition of collagenous or non-collagenous extracellular matrix (ECM) compartments in organs and tissues (1). Several chronic inflammatory disorders are associated with skin fibrosis as the superior complication, including scleroderma (2), rheumatoid arthritis (RA) (3), and systemic lupus erythematosus (SLE) (4). Although collagen deposition is an integral and commonly reversible feature of the wound healing process, the common tissue repair strategy can unwind into a progressively irreversible fibrotic response if the tissue injury is severe and redundant or if the wound healing process evolves dysregulated (5). "Dermal fibrosis" (inflated skin scarring) is an outcome of a bloated healing process, mainly the overblown of proliferation in fibroblasts and production of Extracellular Matrix (ECM) in the dermis. The clinical manifestations of skin fibrosis are mostly thickened, shrunk, and solidified areas of skin. Skin fibrosis may eventually lead to dermal contractures that affect the capability of bending or unfolding the joints. (6). Likewise, fibrosis may influence tumor aggression and metastasis, chronic graft rejection (CGR), and the pathogenesis of numerous advanced myopathies (5). The final stages of scleroderma in human skin involve additional collagen deposition in the dermis with failure of adnexal arrangements and connected adipose tissue (7).

The animal models are essential means widely employed to scrutinize the crucial characteristics

of the diseases that otherwise cannot be investigated at a distance within the human samples (8). They allow the exploration of genes or therapeutic targets without initial endangerment to humans. Rodents' physiology, anatomy, genetic composition, and even behavior is similar to humankind, while above 95% of the mouse genome is identical to humans (9). Due to the accelerated lifespan of rodents, their small size, demanding smaller space to maintain, and the cost-effectiveness of their breeding, many disease models have been developed based on these animals, and diverse reagents have been developed for their application (10). Not enclosing all the animal models utilized to investigate the pathogenesis of skin fibrosis, the current review article (Table 1) focuses on the benefits and impediments of some of the more generally habituated and a number of the lately designed models. Nevertheless, the construction of scars and pathological fibrotic conditions result from multiple pathways working together. Some of the represented models provide suitable options to study distinctive pathways in detail or even the function of unique molecules during fibrosis (11).

Inducible models of skin fibrosis

Animal models that induce fibrosis in the mice are of particular significance because they authorize researchers to inspect the initiating occasions of fibrosis and the possibility of studying several mechanisms. Accordingly, these inducible models have been demonstrated to be immensely practical. However, few chemical compounds can induce skin fibrosis in mice, and sclerodermatous graft versus host disease (GVHD) is a significant challenge while working with these models (12).

Bleomycin induced model of skin fibrosis

Initially sequestered from *Streptomyces verticillus*, Bleomycin is a chemical agent used in cancer chemotherapy and has evolved into a potent initiator of tissue damage and fibrosis induction in mice and different animal species. It can induce both dermal and lung fibrosis models and is suitable for studying SSc-related complications and mechanisms in a system (13). Repetitious administration of bleomycin (the

number of repeats varies in different protocols) may induce skin fibrosis limited to the location of the inoculated zone. Several protocols have been suggested to generate bleomycin-induced dermal fibrosis (14). In general, injecting 100 mg of bleomycin subcutaneously into the shaved locations of the mice's skin daily for 1–4 weeks may induce local skin fibrosis. The magnitude of fibrosis in the bleomycin-induced model relies on the treated mice's genetic background, gender, and health conditions (11). Although the detailed

mechanisms by which bleomycin generates dermal fibrosis are undefined, several research studies declare that bleomycin can perturb cell cycle progression by inducing the G2-phase cell cycle arrest, exerting genotoxicity, and cleaving DNA (15). This model is widely employed to study the roles of distinctive inflammatory mediators, molecules, and cellular components, during fibrogenesis and investigating diverse antifibrotic mechanisms (11).

Table 1. Major characteristics of inducible animal models of skin fibrosis

Inducible models	Induction strategy	Characteristics	Vascular Diseases	Inflammatory conditions	Targeted autoantibodies	Advantages	Limitations
Bleomycin-induced skin fibrosis model	subcutaneous daily administration of 100 mg BLEO for 1-4 weeks.	Skin and lung fibrosis in addition to the infiltration of mononuclear immune cells. Elevated anti-nuclear antibodies.	Exist	Reaching the highest levels between days 3 and 5, afterwards reduction occurs.	The intestinal mucosa	The model is appropriate to various strains of mice. The induced skin fibrosis is stable.	Needs time to be established. Severity differs among various strains
Vinyl chloride (VC)-induced dermal fibrosis	Five successive IP injections of VC in a week for three weeks	Fibrosis, high levels of collagen deposition	N/A	N/A	N/A	N/A	N/A
Hypochlorous (HOCl)-induced model of dermal fibrosis	Subcutaneous administration of 100 ml HOCl for six weeks (everyday)	Skin and lung Elevated anti-nuclear antibodies.	Exists in small renal arteries	Detectable in the skin lesions	Elevated levels of DNA topoisomerase I/Scl70	Better investigating the role of ROS and the Alterations of immune response	Chemicals should be handled with care. Needs time to be established.
Growth factor (GF)-induced fibrosis model	Subcutaneous injections days of TGFβ/bFGF or CTGF for three concurrent	Skin and lung in addition to the infiltration of mononuclear immune cells.	N/A	N/A	N/A	Examination of joint effects of growth factors. Rapid induction of enduring skin fibrosis	Restricted to the study of growth factor-mediated mechanisms.
Sclerodermatous GvHD (Scl-GVHD) fibrosis model	Injection of BM or spleen hematopoietic progenitor cells into RAG2-deficient BALB/c mice.	Skin thickening, advanced fibrosis of internal organs. Rapid immune activation, in addition to the dermal inflammation.	Exist	Local and systemic inflammation and cytokine release aberrations.	Elevated levels of DNA topoisomerase I/Scl70	The model is established and systemic diseases are readily studied.	The implementation is complicated and expensive.
DNA topoisomerase I and CFA dermal fibrosis model	N/A	N/A	Not reported	Cytokine perturbations and inflammation peaks at 8 weeks	DNA topoisomerase I/Scl70	N/A	N/A

Abbreviations: CFA, Freund's complete adjuvant; GVHD, graft versus host disease. N/A: no information available or not applicable

Administration of toxic chemical compounds

Vinyl chloride and hypochlorous acid (HOCl) are the most widely used chemical compounds which can induce dermal fibrosis after local administration. An enzyme called Mixed-Function

Oxidase (MFO) metabolizes and activates vinyl chloride, a carcinogenic chemical. The consequential reactive metabolites eventually result in DNA base-pair shifts during transcription or crosslinks (16). In correlation with the scleroderma-like syndrome, it is believed that

these mutations may instruct the production of autoantibodies against specific antigens, such as HLA-DR (17).

On the other hand, HOCl forges the production of Reactive Oxygen Species (ROS), generates lipid peroxidation, triggers posttranslational modifications, obliterates electron transport chains, bleaching heme cofactors, and tissue damage. These procedures can eventually influence persistent inflammation and dermal fibrosis (18). The HOCl-induced mouse models of skin fibrosis are recently designed inducible animal models and provide additional essential understandings of the initiating signals emanated from the ROS and other molecules that promote fibrosis. The hypochlorous acid mouse model of dermal fibrosis is generally induced by replicated intradermal injections of HOCL, which provokes the generation of hydroxyl radicals leading to augmented collagen synthesis in the skin and lung tissues. Furthermore, this model simulates the pathological alterations observed in systemic sclerosis (SSc) and may induce the production of autoantibodies such as anti-topoisomerase antibodies (19). Similar to the bleomycin-induced mouse model of dermal fibrosis HOCL-induced model could help assess various candidate molecules to prevent fibrosis (20).

The animal model of Sclerodermatous Graft-versus-Host Disease (Scl-GvHD)

The transplantation of allogeneic hematopoietic stem cells, also called bone marrow transplantation, is often used as a therapeutic facet against hematologic malignancies. Nevertheless, it frequently results in dermal and systemic complications provoked by a GVHD reaction (21). The current model exhibits augmented levels of collagen synthesis, as demonstrated in systemic sclerosis. The fibrotic transformations are emanated by TGF- β signaling because inhibition of TGF- β may abolish the progression of fibrosis-associated manifestations (22). The Scl-GVHD model of skin fibrosis is widely used to elucidate the initiating characteristics that lead to systemic sclerosis, the methods by which these factors could be managed, and is primarily derived from

the manifestations of dermal fibrosis in patients suffering from chronic GVHD (10). The currently employed two procedures of the Scl-GVHD model generation in mice differ in terms of conditioning the recipient mice before transplantation. In the first method, which is called the standard Scl-GVHD mode, the isolated and prepared Hematopoietic Stem Cells (HSCs) are injected in sub-lethally irradiated BALB/c mice (21), while in the second method, which is called the modified Scl-GVHD model, the injection is performed on immunodeficient recombina-activating gene 2 (RAG-2) mice (23). In order to study the consequences of anti-TGF β or latency-associated peptide treatment on dermal fibrosis, the Scl-GVHD model is utilized (24).

Growth factor (GF)- induced model of skin fibrosis

The administration of Growth Factors (GF) is also a method of inducing fibrosis. In addition to TGF β , basic fibroblast Growth Factor (bFGF) and connective Tissue Growth Factor (cTGF) can enhance collagen synthesis in fibroblasts and act as prominent choices for an inducible model of skin fibrosis (11). The role of TGF β as an essential originator and effective molecule in dermal fibrosis is established (25). The GF-induced dermal fibrosis model has already been examined on Macrophage Chemoattractant Protein-1 (MCP-1)-deficient mice to scrutinize its pro-fibrotic role in fibrosis initiation and expansion (26).

Dermal fibrosis model induced by DNA topoisomerase I and Freund's complete adjuvant

Autoimmunity is a fundamental aspect of systemic sclerosis, and infrequent experimenters have conceived models that explore this distinctive characteristic of its pathology (10). Yoshizaki et al. indicated that subcutaneous injection of DNA topoisomerase I and Freund's complete adjuvant for eight weeks might stimulate fibrosis in the dermis and lung.

It is worth mentioning that Freund's incomplete adjuvant is not capable of inducing fibrosis. The

fibrosis induction in this model is associated with cytokine imbalance, including elevated levels of IL-4, IFN γ , IL-10, TGF- β , and TNF α (27). After implementing this fibrosis model, the Bronchoalveolar Lavage (BAL) fluid was investigated. The researchers discovered the skewed T-cell profile toward Th2 and Th17 in DNA topoisomerase I and Freund's complete adjuvant receiving mice (10).

Conclusion

Animal models are practical tools for investigating the underlying mechanisms, genetic factors, and diverse characteristics required to establish fibrosis. These circumstances or components usually cannot be examined in human patients.

The many results from in vitro and in vivo investigations would help identify new therapeutic targets to attenuate the fibrotic responses. An ideal animal model for skin fibrosis is not present, but several inducible models are available, suitable for exploring different aspects of the disease. Additional description of underlying disease mechanisms will lead to developing more practical skin fibrosis models.

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Conflict of interest statement

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