KEYSTONE SYMPOSIA

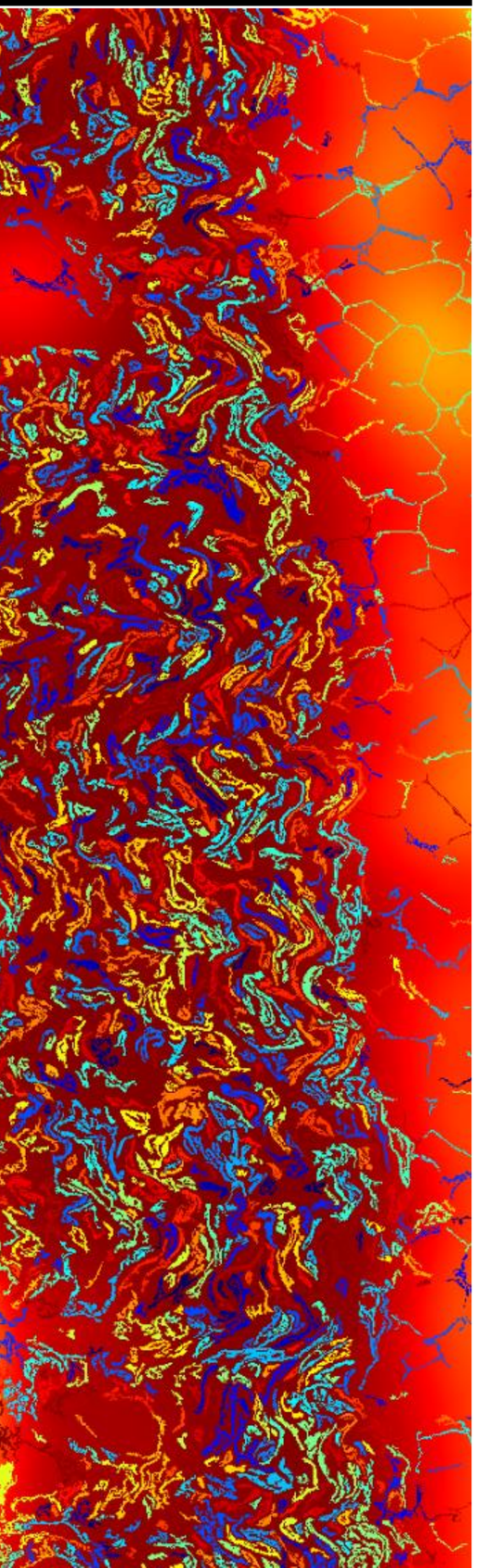
MASH and Fibrosis: From Molecular Phenotypes to Precision Therapeutics
March 2024

Digital Pathology Accurately Quantifies Skin Fibrosis Severity in SPAG17 Gene Knockout Animal Model

Nathan AIST[1], Maria TEVES[2], John VARGA[3], Li CHEN[1]

[1]PharmaNest, Princeton, NJ, USA, [2]Virginia Commonwealth University, Richmond, VA, USA, [3]University of Michigan, MI, USA

PHARMANEST DIGITAL PATHOLOGY



1 Introduction

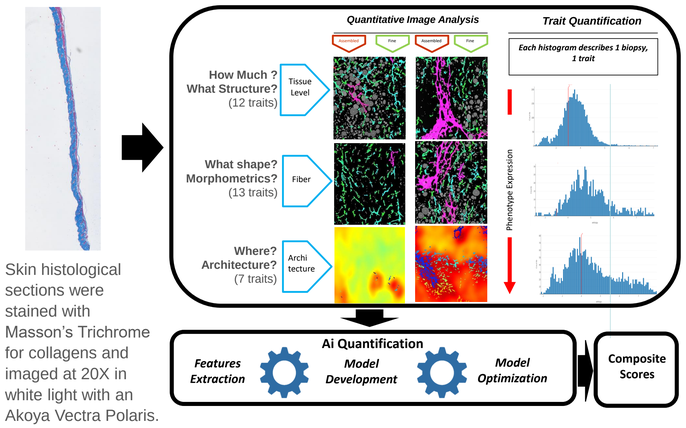
Systemic sclerosis (scleroderma) is an autoimmune disease that primarily affects the skin but may involve multiple organs and tissues. There is no validated animal model or effective treatment for scleroderma. The cilia-associated protein SPAG17 is implicated in the development and progression of scleroderma, with higher disease severity linked to reduced expressions of the gene. We recently observed that mice lacking SPAG17 spontaneously develop fibrotic changes in the skin and other organs.

2 Aim

Here we aim to investigate the application of digital pathology image analysis to quantify fibrosis in the skin from **SPAG17 Knockout (KO)** and **Wild-Type (WT)** control mice. Single-fiber quantitative image analysis (FibroNest™, Princeton, USA) was used to measure the quantity and severity of fibrosis in the dermis and intradermal fat layers.

3 Method

Skin samples were obtained from ten mice matched for age and sex: SPAG17 KO genotype group (n=5) and WT control (n=5). The samples were stained with Masson's Trichrome for collagen and imaged at 20X via Akoya VectraPolaris. FibroNest™, a cloud-based image analysis platform, was used to measure and quantify the fibrosis phenotypes. Average skin thickness was also measured in the two groups.

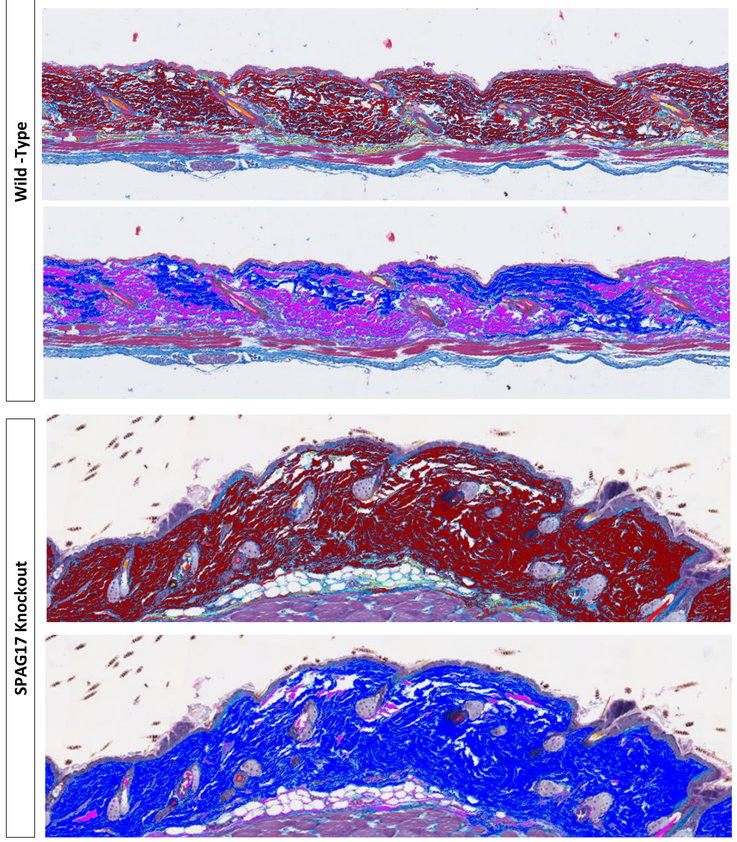


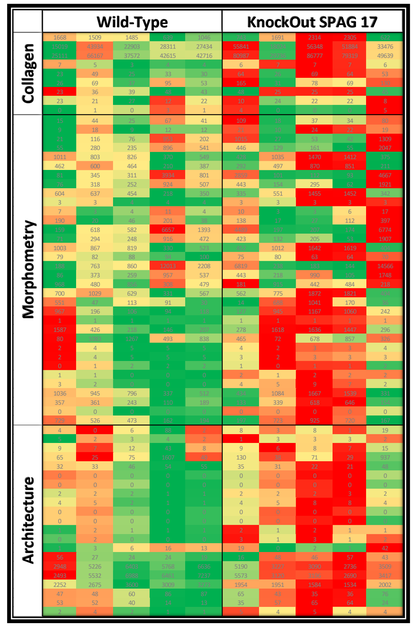
Skin histological sections were stained with Masson's Trichrome for collagens and imaged at 20X in white light with an Akoya Vectra Polaris. Fig. E shows skin histology with mask overlay.

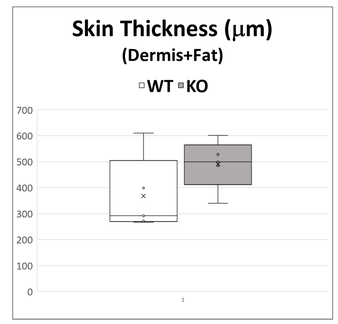
- FibroNest™, a cloud-based image analysis platform, was used to quantify the fibrosis phenotype including 32 traits for collagen content and structure, fiber morphometry, and architecture (measures the organization of the fibers).
- Principal quantitative fibrosis traits (up to 315 qFTs) are automatically detected and combined into a Phenotypic Composite Fibrosis Score (Ph-FCS).
- Collagen quantity and structure, fiber Morphometry (fiber shape and size), and Architecture (organization and proliferation of complex fibers) were measured across 32 quantitative traits.

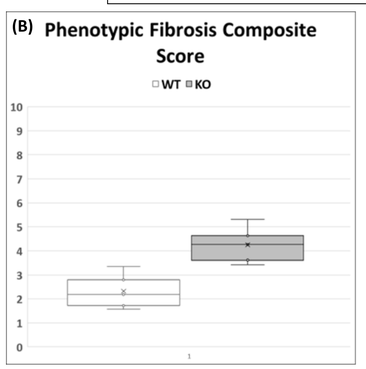
4 Representative Images and Results

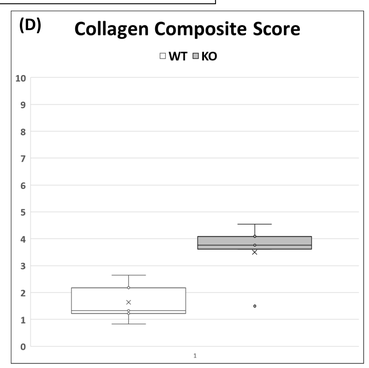
(A) Representative histologic images of skin with FibroNest analysis overlay. SPAG17 KO exhibited higher fiber assembled/fine and collagen reticulation as compared to Wild-Type.











Skin from SPAG17 KO mice exhibited significantly higher collagen content and structure, more complex fiber morphometry, and fibrosis architecture consistent with greater disease severity, demonstrated by the higher PH-FCs score: KO and WT were 4.25 and 2.32, respectively (p=0.0038) (Fig. **B**). Phenotypic Heat chart. Fibrosis severity increases from green to red. (Fig. **C**) Skin from SPAG17 KO mice had higher collagen content overall: the collagen composite score for KO and WT were 3.50 and 1.64, respectively (P=0.0397) (Fig. **D**). SPAG17 KO mice exhibited thicker dermis-fat layers: the KO group average was 491 µm² and the WT group was 368 µm² (Fig. **E**).

5 Conclusions

- Digital pathology image analysis of the skin can accurately quantify fibrosis severity in KO mouse model.
- This technology may play a key role in quantifying the effects of various pathogenic variables related to the development and severity of scleroderma.
- Further investigation into the role of SPAG17 and other cilia-associated proteins may yield promising avenues of inquiry into the possible treatment of scleroderma.

6 Contact information:

nathan.aist@pharmanest.com and li.chen@pharmanest.com