DPP-IV mediates Wnt-induced skin fibrosis

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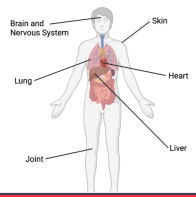
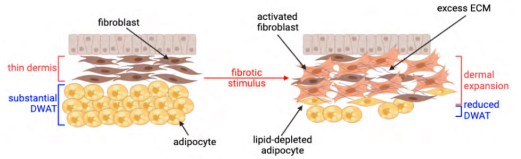
Fibrosis can occur in all soft tissues of the body



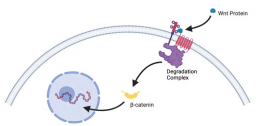
- Fibrosis can be thought of as uncontrolled scarring
- When you get a wound, your body heals itself by depositing large amounts of extracellular matrix (ECM)
- Fibrosis involves the deposition of extracellular matrix in all tissues as well as the loss of fat cells or adipocytes in some tissues.
- Fibrosis can affect all soft tissues, impair organ function, and contributes to approximately 45% of all deaths in Europe and North America[1].
- In the Atit Lab, we study skin fibrosis because of the distinct fibroblast and adipocyte layers which allow us to make connections to other types of fibrosis

Dermal fibrosis involves the deposition of excessive ECM and the loss of fat cells



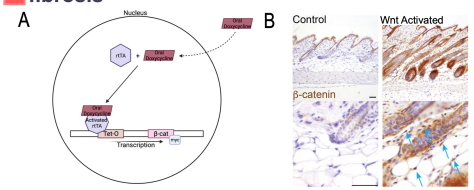
- Skin is composed of the epidermis and the underlying dermis. The dermis is further divided into an upper fibroblast layer and a lower layer of mature adipocytes known as the dermal white adipose tissue (DWAT).
- Intradermal adipocytes contribute to various integral skin functions such as thermoregulation and immune cell recruitment[2,3]. Their ability to perform many of their functions is influenced by their stored lipids.
- Upon introduction of a fibrotic stimulus, early loss of intradermal adipocytes via unknown mechanisms during fibrosis disrupts the DWAT's homeostatic functions. Dermal thickening occurs as increased fibroblast activation and proliferation lead to excessive ECM protein deposition. These events lead to skin dysfunction and morbidity.

Wnt signaling and dermal fibrosis



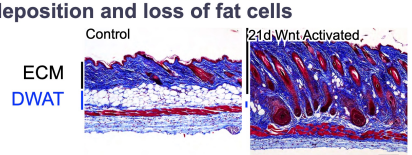
- The Wnt signaling pathway is a well known pathway responsible for the growth and development of many tissues.
- The pathway results in the stabilization of β-catenin intracellularly, resulting in the transcription of specific target genes, including profibrotic genes
- In the skin, Wnt signaling is homeostatically used to control skin patterning and differentiation of different stem cells

Wnt signaling is sufficient to cause dermal fibrosis



- In order to induce fibrosis through Wnt, we use an inducible, reversible genetic model consisting of three transgenes. A) A Cre recombinase is produced, which promotes production of reverse tetracycline transactivator (rtTA). Upon feeding our mice with doxycycline, the rtTA forms a complex with doxycycline resulting in the transcription of stabilized B-catenin
- B) β-catenin immunohistochemical (IHC) staining reveals that nuclear β-catenin is expressed in a greater proportion of dermal cells following Wnt activation relative to control skin. Scale bar=100μm.

Activation of Wnt signaling results in ECM deposition and loss of fat cells



- Over-expression of the Wnt pathway in skin results in dermal fibrosis, resulting in the loss of the DWAT and thickening of the dermis.
- These changes result in loss of mechanical function of the skin, loss of the skin's thermoregulating properties, and reduced immune functionality of the skin. Scale bar=100μm

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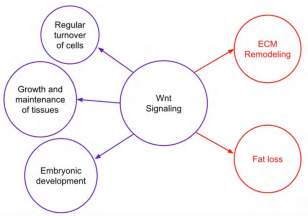
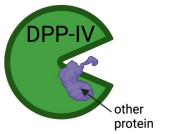
Mediators of fibrosis need investigation to find therapies for fibrosis



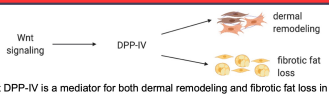
- Wnt signaling cannot be inhibited because of its roles in normal tissue function
- No therapies can effectively reverse both the ECM and fat loss aspects of fibrosis
- As such, downstream mediators of fibrosis need investigation to find therapies for fibrosis

Dpp4 is a likely target downstream of Wnt activation

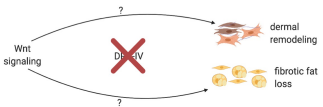


- In a screen of fibrotic proteins, the Atit Lab identified dipeptidyl peptidase 4 (DPP-IV) as a possible mediator of both fibrotic fat loss and dermal expansion
- DPP-IV is transcriptionally regulated
- DPP-IV has been implicated in fibroblast activation and metabolic fat loss before
- DPP-IV already has FDA-approved inhibitors for its role in diabetes, suggesting therapies for fibrosis may be within reach

Hypothesis: I hypothesize that DPP-IV promotes Wnt-stimulated dermal remodeling and fibrotic fat loss in skin fibrosis.

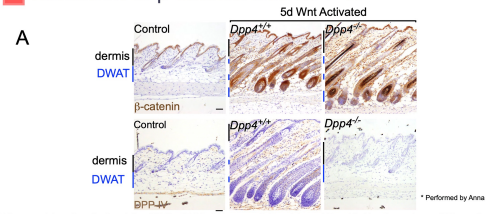


- We propose that DPP-IV is a mediator for both dermal remodeling and fibrotic fat loss in dermal fibrosis



- We tested this by using our Wnt-inducible model bred into a *Dpp4*$^{-/-}$ mouse and then asking the question of whether we still see dermal remodeling or fibrotic fat loss

Validation of β-catenin and DPP-IV

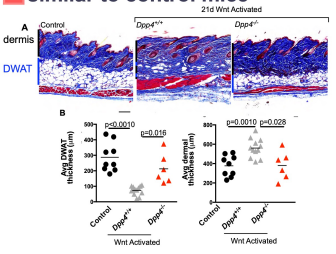


A) Immunohistochemical staining (IHC) of DPP-IV in control, *Dpp4*$^{+/+}$, and *Dpp4*$^{-/-}$ skin after 5 days of Wnt activation. Results indicate that in both control and *Dpp4*$^{+/+}$ mice, DPP-IV is present, while in *Dpp4*$^{-/-}$ mice, DPP-IV is not present. Scale bar=100μm

* Performed by Anna

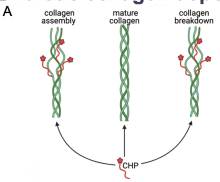
Dpp4$^{-/-}$ mice are protected against Wnt-induced skin fibrosis

Dpp4$^{-/-}$ mice retain dermal and DWAT thickness similar to control mice

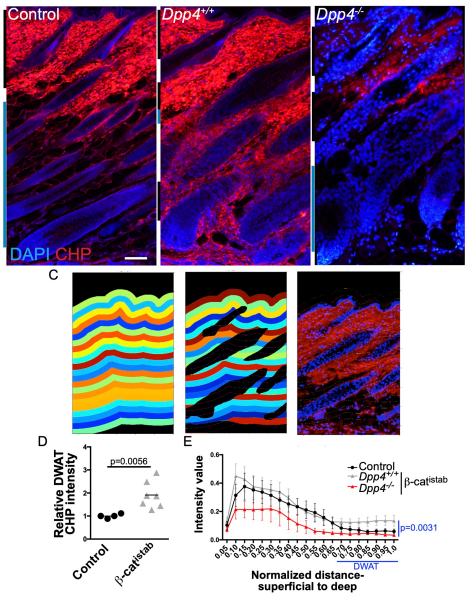


A) Masson's Trichrome stained images of control skin, skin at 21 days of sustained Wnt activation, and skin of Dpp4$^{-/-}$ mouse at 21 days of sustained Wnt activation. B) Quantification performed in FIJI of dermal and adipocyte layer thickness of control, Wnt activated, and Dpp4$^{-/-}$, Wnt activated mice. Scale bar=100μm.

Dpp4$^{-/-}$ mice are protected from Wnt-induced fibrotic collagen deposition

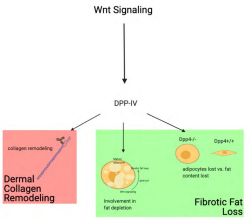


- Collagen is the main ECM protein in skin and is made up of 3 strands twisted in a triple helix
- Collagen Hybridizing Peptide (CHP) binds to collagen that is in the process of winding or unwinding
- It has a red fluorescent tag and when it attaches to collagen, it lights up



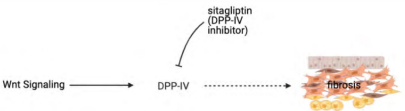
A) Schematic depictind how Collagen Hybridizing Peptide attaches to collagen. B) Collagen Hybridizing Peptide (CHP) staining of Control, 10d Wnt-activated *Dpp4*$^{+/+}$, and 10d Wnt-activated *Dpp4*$^{-/-}$ mouse skin. CHP stains for remodeling collagen and serves as a marker of collagen deposition in fibrotic onset. 10d Wnt activation leads to elevated CHP staining intensity in the DWAT which is rescued by *Dpp4* knockout in *Dpp4*$^{-/-}$ mouse skin. Scale bar=25μm. C) Images from pipeline developed and used to quantify CHP intensity. D) Quantification of CHP Intensity from the DWAT LayerE) Quantification of CHP as distance from the epidermis increases.

Summary



- Dermal Wnt signaling activation is sufficient to cause fibrosis including dermal expansion and fibrotic fat loss.
- *Dpp4*$^{-/-}$ mice are rescued from Wnt-induced DWAT lipodystrophy, adipocyte lipid droplet size, and collagen deposition.

Future Directions



- Since genetic deletion is not an effective therapy for fibrotic disease, investigation of the effects of FDA approved DPP-IV inhibitors, like sitagliptin, is being done.

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